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# TEXT-BOOK

*of*

# BACTERIOLOGY

*By*

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# PREFACE

In this text-book of bacteriology an attempt has been made to give complete and accurate descriptions of the micro-organisms commonly encountered in medicine, comparative pathology, and hygiene and public health. To select from the countless species described in the literature of bacteriology those particular organisms which should be retained and made the basis for future investigations has proved to be a discouraging and almost hopeless task. The selection has been made to a large extent upon my personal observations on material from many different sources, clinical cases, autopsies, intestinal contents in health and disease, samples of soil, water, milk, and food. This work was started some years ago in the Molson Pathological Laboratory of McGill University when I was working under the late Professor J. G. Adami, and was continued from time to time in the Pathological Laboratory of the Johns Hopkins University under Professor W. H. Welch. To both Dr. Welch and Dr. Adami I am under lasting obligations for the opportunities which made this work possible. A considerable amount of material was obtained at the Department of Animal Pathology of the Rockefeller Institute, at Princeton, through the courtesy of Dr. Theobald Smith. During the progress of this work a very large number of cultures have been investigated and complete records kept of their morphology, cultural reactions, and pathogenic properties. These descriptions have served as a foundation for the section dealing with systematic bacteriology.

It is manifestly impossible for a single individual to command more than a small part of bacteriology so widely has this source developed along new lines during the past few years, and in consequence the majority of the descriptions have been taken from the literature. Whenever it has been possible the original statements of the authors who established the species have been followed. When these were evidently incorrect or inadequate those subsequent descriptions have been utilized which seemed to indicate that the investigators were working with the species previously established. Finally many of the descriptions are composite in the sense that they are made up of statements made by many different bacteriologists dealing with the same species. The authority for the descriptions and for the separate statements has been indicated in the text.

This text-book is not intended to present systematic bacteriology in its entirety. Indeed, it is doubtful if any system of bacteriology could be made satisfactory in the present state of confusion. In certain particulars, however, an attempt at completeness has been aimed at. Thus especial emphasis has been laid on the descriptions of the pathogenic organisms responsible for disease in man and animals. The anaërobic bacteria, which have proved to be of such importance in infectious processes or which are responsible for many of the decompositions of milk and foods, have been reported at some length. The principal aërobic spore-bearing species have likewise been included, chiefly because of the importance they possess for the modern hygienist. An attempt has been made to give an adequate treatment of

our present knowledge of the spirochetes. The significance of these organisms in disease production and the lack of any satisfactory discussion of them in current text-books of bacteriology are ample justification for this endeavor. For much the same reasons the infectious agents of undetermined character, the so-called filtrable viruses, have been considered in detail.

Since this book is intended primarily for the medical student and the medical bacteriologist, the methods and technic of bacteriology have been included. The subject of infection and immunity has been discussed with the idea of outlining our present views as they explain infectious processes, rather than with the purpose of giving technical methods which are now adequately presented in a number of American publications.

Throughout the preparation of this book I have constantly made use of current text-books and monographs. In the treatment of infection and immunity I have followed the authoritative papers of the late Professor Wassermann, published in the Handbuch der pathogenen Mikroorganismen of Kolle and Wassermann; in discussing disinfection, the papers of Dr. Gotschlich and Dr. Bürgi in the same treatise.

The problem of preparing suitable illustrations for bacteria has been especially difficult to solve. It was finally decided that careful free-hand drawings give details of structure which are impossible to obtain with photographs. These drawings have been made with a Bausch and Lomb microscope, 12.5 ocular, 1/12 fluorite oil immersion lens, tube length 160 mm., magnification 1260 diameters. The size has been determined by projection with a camera lucida and the drawings have practically all been brought to the same scale. It is at all times difficult to obtain trustworthy cultures of bacteria, and a large number of drawings have been made of strains whose identity has been carefully determined. Only a part of these illustrations has finally been considered suitable for reproduction and I am greatly indebted to Mrs. Edna Miller Rever for the skill which she exercised in making them.

The members of the department of bacteriology have been untiring in their help, have read over many of the chapters carefully, and have made valuable suggestions as to the presentation of the material. I am under especial obligations to the late Dr. P. D. Meader, Dr. Linda Lange, Dr. S. R. Damon, and Dr. W. A. Feirer. Dr. Veader Leonard has carefully criticised the chapter dealing with the destruction of bacteria; Dr. C. G. Bull and Dr. R. R. Hyde of the department of immunology, the chapters on infection and immunity. The bibliography has been verified by Miss Harriet A. Blogg.

WILLIAM W. FORD.

BALTIMORE, MD.,
*June*, 1927.

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# TEXT-BOOK OF BACTERIOLOGY

## PART I

## GENERAL BACTERIOLOGY

### Chapter I

### HISTORICAL INTRODUCTION

The bacteria (Gr. *βακτήριον*, a little stick) include a large number of highly diversified microscopical organisms which are grouped among the plants partly because of their morphology and chemical composition and partly because of their biological reactions. The science of bacteriology may be said to have originated in the attempts of various observers to solve two obscure problems in nature—the origin of life and the origin of death. In the attempts at solution two great controversies arose, one concerning the spontaneous origin of life, the other concerning the communicability of disease. These controversies furnished fruitful topics of observation, experiment, and discussion for several centuries, and were eventually settled only when two instruments of precision, the simple and compound microscope, were utilized in the study of natural phenomena. From the application of microscopes to the study of living material the science of bacteriology came into being, and nearly every great advance in bacteriology was associated with and made possible by some improvement in the construction of these instruments and some advance in microscopical technic. We can, therefore, best appreciate the historical development of the science of bacteriology if we preface this consideration with a discussion of the microscope and then indicate how the various speculations about the origin of life and of disease were eventually settled by its use.

### THE SIMPLE MICROSCOPE

Simple lenses, or magnifiers of transparent substances, with one convex surface and one flat surface or two convex surfaces with the same optical axis, were known to the ancient Greeks and Romans and are mentioned in the writings of Pliny, Plutarch, and Seneca. Convex lenses of rock crystal were found by the archæologist Layard in the ruins of a palace at Nimrud below the ancient City of Nineveh in Assyria. Such lenses were probably used chiefly for cutting precious stones and for the manufacture of jewelry. Euclid in the 3d century B. C. investigated the laws of refraction. Seneca understood the principle of magnification and in his *Questiones Naturales*, written about 63 A. D., says that "letters however small and dim are comparatively large and distinct when seen through a glass globe filled with water." Ptolemy of Alexandria, about 153 A. D., also studied the laws of refraction in his mathematical and astronomical investigations.

Much later the Arabian Alhazen (Abu Ali Al-Hasan Ibu Alhasan 965–1038 A. D.) worked out the optical properties of curved mirrors, and Niblo of Poland in the first half of the 17th century investigated the refraction produced by air, water, and glass. Simple lenses were familiar to Roger Bacon (1214–1294), who cleared up many of the laws of reflection and refraction and suggested the use of lenses for improving vision. Bacon, indeed, is sometimes called the "Father of Microscopy." Convex spectacles were actually discovered, however, by Salvino d'Amato degli Amata of Florence and by Allesandro de Spina of Pisa, about 1300 A. D., and were familiar to the people of the 14th, 15th, and 16th centuries. Leonardo da Vinci (1452–1519) studied the optical properties of lenses and developed the camera obscura. A little later Maurelico the mathematician (1494–1575), in his turn, investigated the properties of simple lenses.

In 1592 an illustrated book was published at Frankfort with the name of George Hoefnagel (1545–1600) on the title page. This book had copper plates showing drawings of natural objects done by his son Jacob which evidently were made with a magnifying glass.

Simple lenses were also quite familiar to people during the Thirty Years' War (1618–1648).

Possibly the earliest simple microscope in which lenses were attached to crude stands was described by Decartes in 1637 in his *Dioptrique*. This instrument had a magnifier and a concave mirror with the concavity toward the object for purposes of illumination. Hooke in his *Micrographia* (1665) described simple microscopes with lenses of small globules made by fusing long-drawn-out threads of spun glass. Similar glass globules were employed in microscopes by de Torre in Naples.

Kircher (1671) made a crude simple microscope by attaching the object studied to a stage with a kind of rest. He seems to have been the first to employ a microscope for the study of living material, finding "worms" in the blood of patients with bubonic plague, now usually interpreted to have been the rouleaux of red blood-corpuscles. Kircher's microscope gave a magnification of about 32 diameters.

The highest development of simple lenses or the simple microscope came at the hands of the Dutch linen draper, Antony Leeuwenhoek, of Delft (1632–1723). He perfected the manufacture of lenses of short focal distance by grinding them with diamond dust, fastened them to stages, and made remarkably effective instruments. He manufactured a large number, owning 419 lenses and 247 microscopes and presenting several of his instruments to the Royal Society of London. According to Charles Singer, who has examined and tested these instruments, their highest magnification is about 160 diameters. Leeuwenhoek made 26 other simple microscopes with a magnification varying from 40 to 133 diameters.

With his simple microscopes Leeuwenhoek examined all sorts of objects and made discoveries which are the foundation of several of the natural sciences. His writings were in the form of 375 letters to the Royal Society of London and 27 papers sent to the French Academy of Sciences. Among other things he observed crystalline matter in tea, coffee, and pepper, the cellular structure of wood, the structure of teeth, bone, and voluntary muscle, the epithelial scales of the skin and mucous membrane of the mouth, the blood-vessels and coats of the intestine, and the structure of the smaller blood-vessels. Of living things he saw spermatozoa, infusoria, various protozoa, including the liver fluke and the larger bacteria. In 1675 he discovered animalcula in rain water, sea-water, spring water, pepper decoctions, and in the discharges from the intestinal canal of horses, doves, fowls, frogs,

and flies. In 1683 he observed a great variety of such forms in the material collected from the interstices between the teeth, diluted or mixed with a drop of saliva or rain water. Leeuwenhoek found that many of these microscopic forms were actively motile, and this observation, together with the drawings which he made, leaves no doubt that he actually saw the larger bacteria in these various materials. Leeuwenhoek's work, indeed, may be regarded as the foundation of bacteriology, protozoölogy, and embryology. Following the work of Leeuwenhoek simple microscopes were used for years and are still employed where high magnification is not essential, as in the dissecting microscope of every laboratory. It is interesting to note that Charles Darwin took a simple microscope with him on the voyage of the Beagle in 1872.

## THE COMPOUND MICROSCOPE

Microscopes consisting of a system of lenses for magnification instead of a single lens as employed in the simple microscope came into use toward the latter half of the 16th century or the first half of the 17th. Their discovery is intimately bound up with the discovery of the telescope. Leonard Diggs, who died in 1571, is said to have constructed the first bilenticular system. Gianbattista della Porta (1540–1615) actually combined lenses in the form of a microscope, and in his *Magia Naturales*, published in Naples about 1588, refers to the use of such an instrument. The discovery of the compound microscope is usually attributed to the Dutchman, Johann Janssen (1580–1616), and his son, Zacharias, of Middelburg, Holland. Zacharias Janssen was a spectacle maker who accidentally observed that two lenses put together in a system give a higher magnification than a single lens, the basic principle of the compound microscope and the telescope. The Janssens made microscopes before 1619, the first being constructed, apparently about 1590, of strong biconvex lenses and a negative eyepiece. It is well known that Cornelius Drebbel of Alkmaar brought a kind of microscope or telescope made by the Janssens to England in 1619 and showed it to William Borell, Dutch Ambassador to France and later to England (1591–1668). This instrument consisted of a set of lenses in a copper tube about 6 feet in length and 1 inch in diameter. Jan Lippershey of Wessel also claimed the invention of the microscope about 1608. The invention of the telescope is attributed to James Metius of Alkmaar.

Such crude instruments may be regarded as the outcome of more or less accidental discoveries by men who were engaged in the business of making lenses. The effective scientific discoverer of the compound microscope and telescope is probably Galileo (1564–1642). He studied the laws of the refraction of lenses put in a system which he said was quite different from just putting two lenses together and noting the higher magnification which results. He is said to have constructed a compound microscope about 1609.

The instruments designed by the Janssens were the actual forerunners of the modern microscope. Fontana in 1646 greatly improved Janssens' microscope, substituting a positive eyepiece for the negative and getting a magnification much greater than obtained by Janssens' instrument. This type of microscope evidently remained in use for some years, for Otto Friedrik Müller (1730–1784) employed an instrument designed by the Janssens and improved by Fontana which is said to have given a magnification of over 300 diameters. Before this time the highest magnification obtained in any style of microscope was that gotten by Leeuwenhoek, about 160 diameters.

Other compound microscopes known are those of Hooke (1665), Bon-

nani (1697), and Divini, who described a kind of compound microscope before the Royal Society in 1668.

In the beginning of the 19th century a number of improvements were introduced in microscopes which have resulted in our modern instruments. The difficulty in all the older microscopes lay in the chromatic aberration. As lenses are made more nearly spherical with a shorter focus (less than 1 inch) the obliquity of the rays so greatly increases the aberration that false colors are introduced and definition becomes defective. The limitation of the size of the aperture to keep down the chromatic aberration shuts out the light and makes the field too dark for successful vision. Amici of Modena in 1812 made the first attempt at an achromatization of the objectives. Improvements of the same character as those worked out by Dolland for the telescope were introduced, the chromatic aberration of the objectives was corrected by a combination of concave lenses of flint glass with convex lenses of crown glass, while the spherical aberration was done away with by a combination of convex and concave surfaces of different curvatures. Amici had a highly curved plano-convex front lens and cemented pairs of lenses consisting of plano-convex flint lens and a biconvex crown lens with a numerical aperture of 105°. Lister (the father of Lord Lister) in 1830 worked out another principle for the correction of aberration by having the image point of one lens coincide with the object point of another. Selligues and Chevalier of Paris superimposed three or four combinations, each of double convex lenses of crown glass cemented to plano-convex lenses of flint. Thus the principle of the modern high-power objective was established. It is interesting to note that Chevalier in 1830 made his apochromatic system after the designs of Selligues and that three London opticians, Ross, Powell, and James Smith, adopted the principles of the French physicists and made objectives which were better mechanically than the Continental. By the year 1837 excellent microscopes were available giving a high magnification and a clear definition. Amici also discovered the use of water immersion lenses and Andrew Ross in 1839 invented a collar adjustment for them. The water lens was greatly improved by Hartnack in 1855. The first microscopist to use oil instead of water for lenses was apparently Wenham, who demonstrated the use of cedar oil before the Royal Microscopical Society in June, 1870. A little later Stephenson, in 1878, also employed the principle of oil immersion for lenses, and at his suggestion Abbe manufactured the first effective immersion lens in which cedar oil was employed. Many other advances were made in the construction of microscopes about this time, chiefly by Abbe, who profited greatly by Helmholz's investigation on the undulatory nature of light. He made an apochromatic objective in 1880 in which the secondary spectrum was not noticeable and which was used with a compensating ocular made of Jena glass. He also introduced the substage condenser which now goes by his name.

The chief improvements in oculars were devised by Huygens, who manufactured negative oculars with the real image below the field piece and above the other lenses in the ocular. Huygens used a plano-convex field piece and a plano-convex eyepiece, both convexities facing downward.

Since 1880 many improvements have been made in the mechanical construction of microscopes, but no essential change of principle has been adopted. Artificial glass of great variety and excellence has been invented and the natural substance fluorite has been largely employed for the front lens of the objective. At the present time microscopes of great practical utility are manufactured by a number of German, French, English, and American firms.

## SPONTANEOUS GENERATION

The belief that living things are generated spontaneously from non-living material, or the doctrine of abiogenesis, as it is called, was widely held in early times. It is mentioned in the Bible, and Aristotle, 325 B. C., records the contemporary ideas from many different countries as to spontaneous generation. Pliny, 23–79 A. D., refers to the growth of frogs, etc., from the mud of the river Nile and likewise indicated the belief of his contemporaries in this theory, which continued to be held all during the early part of the Christian era and throughout the Middle Ages.

Almost the first observer to record any definite facts against the doctrine of abiogenesis was Francesco Redi of Florence (1626–1679 A. D.). Redi was a man of many accomplishments, poet, linguist, naturalist, and physician. He demonstrated that the worms found in putrefying meat come from eggs laid by flies and thus accounted for one supposed type of abiogenesis. Kircher's discovery of the "worms" in the blood of patients with bubonic plague and Leeuwenhoek's discovery of the animalcula, including the bacteria and protozoa, reopened the question of abiogenesis from a new angle in the latter part of the 17th century. The first person to apply experimental methods to solve the problem of the spontaneous origin of life was apparently Needham (1713–1784) in the early part of the 18th century. Needham carried out crude experiments in which he placed vegetable infusions and putrefying material in sealed flasks, heated them for a short time by immersing them in boiling water, and later found them teaming with animalcula. He came to the obvious conclusion that living things originated spontaneously from non-living material. About this time Buffon (1707–1788 A. D.) suggested that the death of higher animals resulted in the disintegration of their molecular substance into vibrios, monads, and collections of cells such as the infusoria.

A little later Spallanzani (1729–1799 A. D.) investigated the question. He repeated Needham's experiments with more carefully controlled technic, used glass flasks which could be more thoroughly heated, took greater precautions in sealing them, and reached conclusions diametrically opposed to those of Needham. Spallanzani believed that living material always originated from other living material. Needham met Spallanzani's conclusion by pointing out that the extreme heat employed by the latter might have produced chemical changes in the material as a result of which it would no longer support life and spontaneous generation was thus impossible.

In 1774 Priestly discovered oxygen with its remarkable properties of aiding combustion and supporting life. This discovery opened up a new avenue of reasoning since the flasks of material in which spontaneous generation failed to take place might well have been devoid of the proper oxygen to enable life to generate and go on. From this time the experiments were devised in such a way as to admit a free supply of this life-giving substance.

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In 1836 Schulze repeated the experiments of Needham and Spallanzani, washing the air admitted to the flasks in strongly acid solutions, and found no development of living material in his boiled decoctions. A year later Schwan (1837) got practically the same results, using highly heated air instead of air washed in acid. In both instances objection to their conclusions could still be raised, since the oxygen admitted might be changed in its properties and no longer support life. In 1854 Schroeder and Dusch showed that air could be admitted to the sterilized flasks through filters of cotton plugs and when proper precautions in sterilization were carried out the flasks remained free from micro-organisms. By this time knowledge

of bacteria had been greatly augmented by the work of Müller and of Ehrenberg and the association of microscopic organisms with the fermentation observed in fluids was pretty clearly recognized. Pouchet, finally, in 1859 announced that bacteria would grow in a hay infusion in an atmosphere of pure oxygen even after all the materials used had been thoroughly freed from living matter.

Pouchet's fundamental experiments may have been correct since the methods available to him for the destruction of living matter would not suffice to destroy spores. His conclusions were so opposed to the generally accepted belief in the doctrine of biogenesis, strengthened as it had been by the discovery of the yeast cells and various types of bacteria in pathological conditions, that attention was again centered on this problem.

In 1860 the French chemist Pasteur undertook an investigation of the phenomena of spontaneous generation and finally settled the question by a series of experiments carried out over a number of years. For the cotton plug of Schroeder and Dusch he substituted a bent tube through which air was admitted to his flasks and at the same time demonstrated the presence of micro-organisms floating about in the atmosphere. He showed that the deposit at the bend of the tube conducting the air into his flasks contained numerous bacteria and that if this were allowed to wash into the flasks fermentation of the fluids resulted. Finally he showed that some of this deposit of air organisms could be introduced into fresh flasks of sterile material and would induce putrefaction. This may be regarded as the most important of his contributions in this field. While previous workers had been mainly concerned in devising experiments to show that fermentation failed to develop when the infusions were sterilized and air admitted in such a way as to exclude micro-organisms, Pasteur not only did this, but showed that these micro-organisms were definitely the cause of the changes which took place in his sterilized solutions and observed their actual multiplication by microscopical examination. In addition, Pasteur showed that sterilization is not always effected by boiling the fluids and that certain bodies, called "corpes brillantes" by him and now known to be bacterial spores, require a high temperature for their destruction.

Bastian in 1872 may be regarded as the last important exponent of the doctrine of abiogenesis, but his experiments were soon shown by Pasteur to be faulty.

From this time on the doctrine of biogenesis, that all living material comes from other living material, and that the atmosphere is the source of the micro-organisms which give rise to fermentation and putrefaction, was almost universally held. Tyndall indeed in 1876 gave indirect confirmation of this by showing that optically pure air is free from bacteria. The great practical result of this phase of Pasteur's work is not so much that he finally settled the controversy regarding spontaneous generation, but that his observations on the pollution of the atmosphere by bacteria paved the way for Lister's antiseptic surgery which has revolutionized surgical practice throughout the world. Acting on the theory that suppuration in operative wounds is directly due to micro-organisms introduced from the air, Lister devised methods by means of which these micro-organisms could be destroyed. As a result of these methods it became possible to avoid the usual suppuration in wounds and the era of antiseptic surgery was inaugurated. It is interesting to note that Lister employed carbolic acid because of its use in the destruction of sewage in the little town of Carlisle, England. Even before this time the French apothecary Lemaire had studied the properties of carbolic acid and had shown that the addition of minute

traces to fermenting fluids stopped the growth of micro-organisms. Lemaire's work on carbolic acid indeed is the foundation of modern work on disinfection.

More modern observations have shown that the atmosphere does not play so important a rôle as Lister believed, since the bacteria in the air are usually devoid of pathogenic action. Indeed, many factors are concerned in the production of wound infections, and bacteria are introduced into wounds not only from the air, but from the hands of the operator, the instruments he uses, the skin of the patient, and possibly from his blood-stream. For our present purposes it is sufficient to emphasize that the work of Pasteur and Lister demonstrated firmly the thesis that the decomposition of fluids and the infection of wounds are the direct result of the growth of micro-organisms, whatever be their source.

## CONTAGIUM VIVUM

In primitive times people held what we now regard as primitive ideas as to the source of disease. Considering the state of knowledge of the causation of natural phenomena in general, we can hardly be surprised that disease should be ascribed to supernatural forces and particularly to the influence of an all powerful Deity or to individual gods who were endowed with special powers. Such beliefs are still held among savage tribes, and the American Indians up to within recent times attributed sickness and death to the wrath of the Great Spirit. Nor is the belief that disease results from great meteorological changes, floods and earthquakes, any the less unnatural since devastating pestilences are by no means uncommon after profound alterations in the character of the earth's surface. In modern times even well-trained medical men have held such notions, and the great pioneer physician, Daniel Drake, in his little book on *Epidemic Cholera*, suggests the action and influences of meteorological forces, particularly great changes of weather, as etiological factors in the epidemic of cholera which visited Cincinnati in 1838.

Very early in the history of the world the conviction was forced upon people that the individuals suffering from disease might themselves be the source of the same disease in others. As was to be expected, those types of sickness marked by outspoken external manifestations were the first to be so viewed, and we find that the idea of contagiousness or communicability of disease first began to be entertained in leprosy and in bubonic plague. The drastic laws passed for the control of lepers, their cruel segregation, the restrictions which they were forced to submit to in procuring food and clothing, and the fear of them entertained by the general populace, so that lepers were compelled to announce their presence by wearing bells or crying out "unclean," all testify to a deeply rooted conviction that leprosy was easily communicated from person to person.

The recognition of the communicability of bubonic plague was also very early. The description of this disease in the reign of Justinian leaves little doubt that the people of that era believed in the ability of the sufferers from plague to pass it on to others. This belief was confirmed and magnified by the frequent visitations of plague upon various parts of the world in the Middle Ages when the Black Death decimated the population of Europe. By the latter part of the 14th century the contagiousness or communicability of plague was well recognized in principle, as is shown by the action of the people of Venice who appointed a council of three to control the disease and later established a quarantine or period of detention of forty days

before travelers from plague-infected ports were allowed to disembark or before goods could be discharged. We find current expression of this belief in the writings of such men as Pepys, who has given us a fine objective description of the Great Plague of London in his *Diary*, and De Foe, whose *Journal of the Plague Year* is now recognized to be an accurate description of the London epidemic of 1665. Boccaccio also in his Introduction to the *Decameron* seems to be thoroughly convinced that plague could travel in some mysterious way from the sick to the well.

Possibly the natural history of syphilis had the greatest influence in teaching the world that disease can pass from individual to individual. Here the mechanism of transmission was almost self-evident, and soon after syphilis appeared in the Old World it was generally believed that one person could catch it from another. When syphilis began to spread widely in Europe after the siege of Naples it is said that a kind of quarantine was established against it similar in principle to the quarantine against bubonic plague.

The growing conviction that diseases like leprosy, plague, and syphilis could be communicated from one person to another led by imperceptible stages to the doctrine that disease is due to some mysterious kind of living principle which the sick transmit to the well, and by the middle of the 16th century we find this doctrine pretty well expressed although by no means universally accepted. In 1546 Fracastorius first clearly expressed the idea of the communicability of disease. He divides the diseases which he regarded as contagious into two kinds—those transmitted by contact of person to person, *per contactum*, and those transmitted by objects in the external world, *per fomitem*.

Possibly the Latin poet Lucretius was the first person to suggest that disease is caused by minute living things which he called "animalcula." His suggestion was never taken seriously and is of interest chiefly in that the term "animalcula" came later to have great significance. It was not till a century after Fracastorius that Athanasius Kircher with his crude lenses observed his peculiar "worms" in the blood of patients with bubonic plague. While we know now that these were not living agents, but probably the rouleaux of red blood-corpuscles, Kircher's observations were of great importance since they gave something tangible to discuss, and from this time on the animalcula came to have a great importance in all the speculations in regard to the origin of disease.

A little later Leeuwenhoek's discovery of animalcula in the tartar about the teeth, in various excretions of the body, in rain water, etc., suggested a ready explanation for those who believed in some form of animate contagion, but the properties of the bacteria were so obscure at that time that little wonder can be felt that their significance in disease production was not at all appreciated. It was not indeed till the middle of the 18th century, when discussion of spontaneous generation became more general, that the doctrine of "contagium vivum" was more or less definitely put forward as a theory to account for disease by the Vienna physician Plenciz, who in 1762 held that each malady had its own specific agent which multiplied enormously in the body and gave rise to the lesions and symptoms. Plenciz seems to have been the first to recognize the possible significance of Leeuwenhoek's discovery of the bacteria, and on the basis of this discovery, and of his own finding of animalcula in all sorts of decomposing materials, he drew conclusions which were quite unwarranted by his observations. He did, however, first describe the "bread ferment," observing the same type of microscopical organism in some of the original substance and in the dough to which

the ferment was added. From this time on the development of the doctrine of "contagium vivum" is inextricably associated with the controversy regarding spontaneous generation and with the theories about fermentation or decomposition. The celebrated physicist, Robert Boyle, had already in the 17th century suggested that the explanation of disease would eventually be found in the explanation of fermentation. At the same time the slowly developing knowledge of the activities of microscopical forms of life began to be more widely disseminated, and we soon find a crude attempt to explain both "animate contagion" and "fermentation" as a result of the growth of some kind of living organism too small to be seen with the unaided eye.

The knowledge of bacteria was still in its infancy at this time and Plenciz's conclusions were based on hypothetical properties of microscopical organisms about which so little was actually known that the botanist Linnæus put them all together in one group which he named Chaos. In 1786 Otto Friedrik Müller, of Copenhagen, published a systematic classification of the *Animalcula infusoria*, using the compound microscope invented by the Janssens and improved by Fontana which is said to have given a magnification of over 300 diameters. With this instrument he could distinguish the different forms of organisms far more completely than could Leeuwenhoek with his perfected simple microscope. Müller established a crude classification which was a considerable advance over previous work, introducing the terms "Monas," "Proteus," and "Vibrio" with much the same significance that is employed today. The compound microscope was still an imperfect instrument, however, and for the next forty years little advance was made. In the early part of the 19th century the improvements in the construction of microscopes by Amici, Selligues and Chevalier, and others furnished scientists with instruments far superior to those previously utilized and we now begin to see observations which are of permanent scientific value. The year 1837 marked a number of important achievements. In this year Cagniard Latour, Schwann, and Kützing studied independently the yeast cells in fermenting beer and wine previously seen by Leeuwenhoek and named *Mycoderma cerevisiæ* by Desmazières; Bassi showed that muscardin, a contagious disease of the silkworm, was caused by a minute plant inoculating healthy worms with spore material and producing the disease. This yeast cell was subsequently renamed *Torula cerevisiæ* by Turpin. Donné in the same year was the first to find organisms in pathological conditions, observing vibrios in syphilitic chancres and believing them for a time to be the cause of lues.

In 1838 Ehrenberg, with the improved microscopes then available, devised a more extensive and accurate classification of microscopical organisms than that of Müller. The elongated microscopical forms he called Vibriones, making four species, the rigid filamentous Bacterium, the flexuous filamentous Vibrio, the rigid filamentous Spirillum, and the flexuous filamentous Spirochæta. With some minor changes these terms are employed today with the same significance attached to them by Ehrenberg. In the next few years the larger organisms were seen in several of the parasitic skin diseases, the parasites of favus and thrush being observed in 1839.

Despite the few accurate observations this was a period of great confusion since all sorts of structures were described in nearly every variety of pathological condition and regarded as the etiological agents. In 1840 Jacob Henle, the celebrated anatomist, then working in pathology, protested vigorously against the loose reasoning and the ill-founded conclusions then so prevalent. He called attention to the fact that the various structures which had been described as etiological agents of disease were not even

definitely shown to be living organisms, since no methods were then available to distinguish between living and dead material. Henle demanded some convincing evidence that living organisms were constantly present in infectious material and that they really had the capacity to produce the diseases in question. Henle's strong position made a profound impression upon the scientific thought of his contemporaries. The wild and reckless speculation ceased, observations of enduring value began to be made, and conclusions were drawn with the greatest caution from these observations. The use of the microscope had become so universal by this time and it had proved so valuable in elucidating the structure of plant and animal tissues that scientists were everywhere applying it to the study of fermenting or decomposing materials, and to the investigation of the great mystery of disease production. The year 1840 may be said to mark the beginning of the modern science of bacteriology.

In 1840 Felix Dujardin, using microscopes giving a magnification of 300 to 1200 diameters, found micro-organisms in a great many different conditions, especially favus, herpes tonsurans, pityriasis versicolor, and soor, observing both threads and spores in the latter disease. Dujardin also found organisms in the intestinal contents, in typhoid ulcers, in the nasal secretions of glandered horses, and in the membrane of croup. The Scotch surgeon Goodsir discovered *Sarcina ventriculi* in the stomach contents in 1842, this organism being later put by Virchow in the lower plants. About this time various groups of larger organisms were identified, among them being leptothrix, trichophyton, achorion, oïdium, aspergillus, and mucor. There were naturally many imperfect observations mingled with a considerable number which have been verified by modern methods. The organisms were still examined unstained and in fluid preparations, or stained by carmin or indigo.

Pollender in 1849 and Davaine in 1850 described the anthrax bacillus in splenic fever, but hazarded the most conservative conclusions as to its significance. About this time Villemin (1827–92) proved the infectiousness of phthisis by inoculation experiments. The term "virus" also came into general use to signify the potential agent in disease production. In 1853 Delafond produced anthrax in animals, finding the bacilli in the blood and showing that blood could produce anthrax only when organisms were present. No proof was brought, however, that the bacilli were actually the cause of splenic fever.

In the meantime the question of spontaneous generation came to the front again. Schroeder and Dusch showed that a plug of cotton acts as an efficient filter and prevents the entrance of micro-organisms into flasks of fluid sealed with this substance alone. Although the yeast cells, *Torula cerevisiæ*, had already been found by Cagniard Latour, Schwann, and Kützing in fermenting beer and wine, the subject of fermentation was still shrouded in mystery and there was much difference of opinion in regard to the phenomenon. Helmholz, for instance, held that there were two types of fermentation, one accompanied by micro-organisms and another without them. The German chemist Liebig believed that the ferment itself came from dead protein materials. Pasteur approached the problem by the study of lactic acid fermentation. He made up sterile sugar solutions and added portions of the deposit found in solutions of sugar which had turned to lactic acid. This deposit contained organisms quite different from the yeast cell and was termed the "lactic ferment" by Pasteur. With his lactic ferment Pasteur induced a characteristic production of lactic acid from sugar. He next attacked the problem of alcoholic fermentation and

showed that yeast will produce this type of fermentation in solutions containing sugar and non-protein materials. Substances of protein nature are thus not essential for fermentation and Liebig's theory was demolished. Fermentation, according to Pasteur, was always associated with the vital activities of the yeast cells, their development, multiplication, and continued life. As to the actual chemical process by which the sugar is decomposed Pasteur did not at first express any opinion. He subsequently concluded that fermentation is the result of life without oxygen. On the top of fermenting fluids the cells get their oxygen from the atmosphere and do not attack the sugars. At the bottom, in the absence of free oxygen, the cells abstract oxygen from the sugar molecule and secure their growth energy from this decomposition. This abstraction of oxygen results in a splitting of the sugar with the production of alcohol and carbon dioxid, that is, in a fermentation of the sugar. There is thus a fundamental distinction between the aërobic life of micro-organisms in the presence of oxygen and the anaërobic life in its absence. According to Pasteur fermentation and putrefaction are essentially the same process, a ferment being a living organism which can abstract oxygen or other substances from compounds containing carbohydrates or proteins instead of from gases in the atmosphere. Pasteur studied also the fermentation in which butyric acid is produced and showed that the ferments inducing this type of fermentation are different from the lactic ferments.

Following this work Pasteur undertook a study of the problem of spontaneous generation in which he dispelled the doctrine of abiogenesis. He pointed out that the organisms which bring about decomposition in fluids are derived from the atmosphere and that sterilization of fluids requires heat above the boiling-point of water because of the presence of his "corpes brillantes," now known to be spores. These two contributions settled for the time the nature of fermentation and the source of decomposition. Pasteur now turned his attention to some of the great practical difficulties which the French people had encountered in the wine and beer industries. In the course of this work he confirmed his earlier observations on aërobic and anaërobic life and showed that the same species of micro-organism can grow in the air as an aërobe and without the air as an anaërobe. He made important contributions concerning the origin of the yeasts which make wine, finding them outside the grape and only on the ripe fruit. He also solved the riddle of two great diseases of the silkworm, pebrine and flacherie, obtaining practical results which have had the greatest economic value to France, and which in connection with his later work have served to make him the great national hero of that country.

In the period immediately before and after the Franco-Prussian War bacteria were demonstrated in diseased tissues by a number of pathologists. Thus Hallier found them in measles, syphilis, gonorrhea, diphtheria, and glanders, and developed his remarkable doctrine of "pleomorphism," one organism changing to another under different conditions of life. In 1868 Obermeier observed a peculiar spirillum in the blood of patients with relapsing fever. Also von Recklinghausen in 1871 studied pyemia, typhoid fever, and rheumatism, finding bodies with definite characteristics in the lesions. They were organic substances not dissolved by alkalies, acetic acid, or glycerin, and were evidently some kind of organism. In the same year Weigert found bacteria in hemorrhagic smallpox, and Waldeyer in the heart muscle in pyemia and in the kidney in pyelonephritis. Klebs in 1870 and 1871 found bacteria in gunshot wounds, granulation tissue, fistulas, and disease of the bone-marrow. He in his turn developed the doctrine of a

*Microsporon septicum*, a single organism able to produce all sorts of pathological changes. Billroth also in 1874 discovered streptococci in pus, but was not convinced of their etiological significance.

About this time the Botanical Institute of Ferdinand Cohn in Breslau became the great center of interest. Cohn and his co-workers undertook the study of the bacteria as an important and interesting type of plant life, since by this time it was recognized by Cohn that the properties of the bacteria allied them to the plants. Cohn, who had already in 1854 accepted Ehrenberg's classification of microscopical organisms into four groups, Bacterium, Vibrio, Spirillum, and Spirochæta, improved this classification in 1875, making six groups, Micrococcus, Bacterium, Bacillus, Vibrio, Spirillum, and Spirochæta. He first clearly demonstrated spores in *Bacillus subtilis*. Fresenius cultivated the organism producing red pigment, *Monas prodigiosus*, on potato, and this was confirmed by Cohn. This remarkable organism was apparently first observed and cultivated by the Italian Bizio, who named it *Serratia marcescens*. It was subsequently rediscovered in 1838 by Ehrenberg who recognized its bacterial nature by microscopical examination. Hermann Hoffmann in 1869 had cultivated all sorts of organisms on potato and bread. Schroeder in 1875 probably got the first pure cultures of any bacterial species. He cultivated "prodigiosus" on potato, transferring only those portions which gave the red pigmentation. No effective method of separating one species from another, of getting pure cultures, had yet been devised despite the ingenious attempts of Hallier, Klebs, and others. Nor was the microscopic examination of bacteria carried out without great difficulties and many chances of error, the organisms being studied unstained in fluids. Cohn himself appreciated the difficulties of the situation and said that little real advance could be made until the different micro-organisms could be isolated in pure culture, observed over long periods of time, and the development of any particular organism from a pre-existing organism of the same size and shape be traced with certainty. The proof for the thesis that different organisms exist in nature, differing from each other in form, biological reactions, and fermentative activity, a thesis which Cohn regarded as the keynote to the whole science of bacteriology, had not yet been brought.

In 1876 Robert Koch, a country doctor in the little town of Wollstein, published his first paper on the anthrax bacillus, the cause of anthrax or splenic fever in cattle. He had produced artificial infections with the blood of anthrax animals in mice, rabbits, and guinea-pigs, cultivated the organisms in a drop of the aqueous humor of the bullock's eye and in bovine blood-serum on a warm stage, and had observed the development of the organism from vegetative rods to spores and from spores to vegetative rods. He finally produced the disease in animals by his cultivated organisms which were removed from the original blood by a sufficient number of generations to leave no doubt that the anthrax bacilli and not some hypothetical virus were the cause of the disease. For the first time a pathogenic organism was cultivated artificially outside the body in pure culture and the disease produced experimentally with such cultures. In the following year, 1877, Koch published his second paper in which he described the method of making films of bacteria on the surface of glass coverslips and fixing them by drying or gentle heat, and his method of staining them by the anilin dyes, discovered by Ehrlich and first used for bacteria by Weigert, who employed them to demonstrate bacteria in tissues. He also devised a method of staining the flagella or cilia of bacteria, which had hitherto been seen unstained in some of the larger forms, and perfected methods for the photography of

bacteria. He utilized in his work the oil-immersion lens which had been perfected by Abbe and the substage condensor of the latter.

Koch's conclusions on the etiological relationship of the anthrax bacillus to splenic fever were enthusiastically accepted everywhere in Germany. In France they met with much opposition from the physiologist Paul Bert, who maintained that every disease was produced by a virus of the nature of an organized ferment the activity of which was inhibited by oxygen. Since the anthrax bacillus could develop in the presence of oxygen, it could not, according to Bert, be the cause of splenic fever. Bert's opposition to the theory of the bacterial causation of disease led the great Pasteur into the study of anthrax and the infectious diseases, a field in which, so he himself said, "he had thus far been a stranger." Pasteur confirmed all of Koch's observations, cultivating the anthrax bacillus in fluid media and producing the disease experimentally with organisms over 140 generations from the original material. He studied also other infections in animals, discovering about this time the anaërobic *Vibrion septique*, the cause of malignant edema. Pasteur's attention was again focussed upon the analogies between fermentation and disease, and in 1878, in association with Joubert and Chamberland, he threw his great influence in favor of the thesis that all disease is caused by the growth of micro-organisms within the body.

In the same year, 1878, Koch published his work on wound infections in which he had produced artificial diseases in animals by the subcutaneous introduction of material from various sources, these infections being strictly comparable with the wound infections observed in man. Under his direction technical methods for the study of bacteria developed rapidly. In the next few years he himself described his "poured plate method" for the isolation of bacteria in pure cultures, the fundamental principle of all bacteriological research the world over, introduced the use of solid media like blood-serum, gelatin, and agar, and in association with his assistants developed important procedures for the destruction of bacteria by heat and disinfectants. In 1882 he described his results in staining and cultivating the tubercle bacillus and about this time first formulated the rules or postulates which should be met if any micro-organism was to be regarded as the cause of a disease. The organism in question, he said, should first be found in every case of the disease and under conditions which would explain the pathological changes and the clinical symptoms. It should not be found as an accidental or harmless parasite in other diseases. Finally, it should be grown outside the body in pure culture and with such pure cultures the disease should be produced.

Koch's work on the cultivation of the anthrax bacillus, his utilization of solid media, his development of methods for the isolation of bacteria in pure culture, and particularly his discovery of the tubercle bacillus, an organism responsible for about one-seventh of the deaths from all causes, attracted world-wide attention, and soon an active school of bacteriologists developed, trained by him or by his immediate pupils. At the same time the French school of bacteriologists, following the lead of the brilliant Pasteur, made enduring contributions to bacteriology, especially in relation to chicken cholera and to vaccination against anthrax in cattle. The following decade ranks as the greatest decade in the history of medicine, judged by the wealth of enduring contributions to science and by the saving of life in man and animals. One by one the etiological agents of the infectious diseases were discovered. Once the right methods were obtained, these discoveries came, as Koch expressed it, "as easily as ripe apples fall from the tree." Koch himself discovered the cholera vibrio, Gaffky cultivated on solid media the

typhoid bacillus previously seen and cultivated in fluid media by Klebs and by Eberth, Loeffler cultivated in pure culture the diphtheria bacillus apparently first seen by Klebs, and discovered the glanders bacillus, Pfeiffer discovered the influenza bacillus, and Escherich discovered the two most important bacteria of the intestinal tract, *Bacterium coli commune* and *Bacterium lactis aërogenes*. In France Pasteur perfected his methods for vaccination against anthrax and devised a method of vaccination or immunization against rabies or hydrophobia in dogs and wolves. The technical methods for the study of bacteria were greatly improved and apparatus was perfected for various bacteriological procedures. By the year 1890, about fifty years after Henle's challenge to his contemporaries to prove some of their ill-founded claims, bacteriology was firmly established as one of the fundamental medical sciences.

Since the close of that decade bacteriology has undergone a continuous development, but the fundamental methods in vogue by 1890 have not been markedly changed nor have they ever been seriously questioned. The science of bacteriology has been prosecuted in many other fields and important contributions have been made in the dairy industry, in agriculture, in beer and wine making, and especially in solving the etiology of the diseases of plants. Methods have been devised for the rapid identification of diseases in man and animals. The history of the last three decades is replete with discoveries of unquestioned value for the welfare and happiness of the world. These discoveries are so manifold, however, and pertain to so many fields that they can be better understood if they are discussed in the special sections of this book when the individual organisms are described.

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## Chapter II

## MORPHOLOGY OF THE BACTERIA

When Leeuwenhoek discovered the animalcula which we now recognize as bacteria and protozoa the cell had already been observed by Robert Hooke (1635–1703), who used a compound microscope and found small honeycomb-like cavities in charcoal, cork, and plant tissues, and by Malphigi (1628–1694), who had studied various tissues with the cell as the unit. Leeuwenhoek himself observed compartments in the tissues of higher plants and animals, naming them globules. Gradually the conception of the cell as a compartment of a plant or animal containing a number of such structures was modified by the increase of knowledge until Schleiden and Schwann in 1838 formulated the cell theory which teaches that the body is composed entirely of cells and their products, the cell being the unit of structure and function and the primary agent of organization. In 1844 Nägeli showed that cells multiply by division, new daughter cells arising from previous parent cells, our present conception. This idea was converted into a general thesis or doctrine by Remak in 1841 and by Virchow in 1855. Thus Virchow maintained that every cell was derived from a pre-existing cell, "omnis cellula e cellula."

The nucleus was probably first observed by Fontana in 1781 and by Meyen, who saw it in spirogyra in 1826. Robert Brown in 1831–1833 concluded that it is a normal cell element, and he is, indeed, generally regarded as its discoverer. Brown also noted the movement of particles in a fluid which is known as Brownian or Brunonian movement. In 1879 Edward Strassburger announced that nuclei arise from pre-existing nuclei, an observation leading subsequently to the dictum of Fleming, "omnis nucleus e nucleo." The cell contents were described as "Sarcode" in animals by Dujardin (1801–1860) and as "Schleim" or "Protoplasm" in plants by von Moll in 1846. Payen in 1846 and Ferdinand Cohn in 1850 concluded that the sarcode of the animal and the protoplasm of the plant are essentially alike. Later investigations have shown that the "protoplasmic doctrine" probably should take the place of the "cell doctrine," the units of organization being masses of protoplasm of much the same character in all living organisms, the cell wall emphasized by the earlier observers turning out to be of secondary importance. The essential thing is the cytoplasm or the cell contents. The wall is probably a secretion and not a part of the cell proper. Indeed, it may be absent in certain cases, as in the motile cells of the algæ. The bacteria and the protozoa are now regarded as single cells and are frequently spoken of as unicellular organisms as contrasted with the plants and animals which are multicellular and made up of masses of cells. The unicellular forms are often grouped together as the "protista." They differ in important characteristics from the cells of which plants or animals are composed chiefly in that they are not fixed as a part of other structures, but are free to move about and lead an independent existence. They really correspond to the free red and white corpuscles of the blood or to certain free cells in the organs more exactly than to the fixed cells of the body. Their structure can best be made clear by comparing them with tissue cells and indicating how they depart from these in certain essentials.

## THE FUNDAMENTAL MORPHOLOGICAL TYPES

Differences in the form of bacteria were recognized by the earliest observers and the names used to describe these forms have come down to us from past times. The term "bacterium" signifies a rod or little stick and is now utilized for the entire group as well as for the members of a particular genus. The bacteria proper are really elongated cylinders, circular on cross-section. The bacteria in general are also spoken of as micro-organisms, but this term includes the protozoa as well. The bacteria are sometimes called "parasites," but they are not all parasites in the true sense. Not infrequently they are designated "germs," but here again the term is a broad one including the protozoa as well as the bacteria. We may distinguish the following fundamental morphological types:

A. *Spherical Organisms Known as Cocci* (Gr. κοκκος, a sphere).—Such organisms are exactly spherical in their typical vegetative stages, measuring the same in every diameter. They multiply by transverse fission and just before division are apt to be elongated, immediately after division, somewhat flattened. Certain arrangements exist among the cocci according to which they may be divided into different genera, these arrangements depending upon the mode of division. This enables us to differentiate among the cocci certain types as follows:

1. *Streptococci or Chains of Cocci.*—Following the fission of a single coccus into two daughter cocci, a second transverse division occurs in a plane passing through the center and parallel to the original plane of division, subsequent fission always occurring in such parallel planes. As a result chains of spherical organisms develop and the length of these chains depends upon the period of time the adjacent cells remain attached to each other. We thus have long-chained streptococci and short-chained streptococci. Occasionally the two cells arising from the primary division remain discrete and such forms are called *diplococci.* The formation of diplococci is only one event in the formation of chains of cocci, but the term is frequently employed for certain genera in which long chains of firmly attached cocci are not seen.

2. *Sarcinæ or Bunches of Cocci Arranged Like Bales of Cotton.*—Here the first fission is followed by a second fission in a plane through the center at right angles to the original plane and by a third division in a plane parallel to the original first and second. This mode of division results first in two organisms, then in four organisms, and then in eight. This arrangement in eights is retained and one gets a picture of four organisms when the bunch happens to be viewed squarely from one side. Frequently the eights occur in multiples and it may be difficult to make out the sarcina arrangement. Occasionally the original four organisms remain isolated or detached and this form is frequently spoken of as a tetrad. It is generally believed that a tetrad is not a permanent arrangement, but merely one stage in the formation of a sarcina.

3. *Staphylococci or Spherical Organisms Arranged in Bunches or Clusters.*—These were likened by the early observers to bunches of grapes (L. *staphylon,* bunch of grapes). They arise from cleavage of the cells in planes which bear no geometrical relation to the previous planes and the bunch-like arrangement is the result of the organisms remaining attached to each other for some time. The term "staphylococcus" was originally applied to the pathogenic pus-producing cocci and at the present time is so utilized. The term "micrococcus" has much the same significance as the term "staphylococcus," but is used for the non-pathogenic spherical cocci arranged in bunches or clusters, chiefly of environmental origin.

B. *Elongated Cylindrical Organisms Known as Bacteria or Bacilli.*—These organisms are round on cross-section and several times their transverse diameter in length. When looked at from the side they appear as rods, but from the ends as spheres. The term "bacterium" was originally employed for all these cylindrical rods. When spores were discovered by Cohn in the species now known as *Bacillus subtilis*, he differentiated these sporulating organisms from the bacteria and adopted the term "bacillus" as a genus name. Owing to the fact that later authors, notably Migula, employed the term "bacterium" and "bacillus" with a different significance, "bacterium" being used for non-flagellated organisms and "bacillus" for flagellated, considerable confusion has resulted. Strictly speaking, the term "bacillus" should be employed only for those rod-shaped species which produce true endospores, the term "bacterium" for those that fail to produce these bodies. In general, the terms "bacillus" and "bacterium" are used indiscriminately to indicate any rod-shaped cylindrical forms.

C. *Vibrio.*—The term "vibriones" was originally given to the cylindrical organisms curved or bent on themselves and forming a spiral thread or a portion of such a spiral thread. A more exact significance came in Koch's description of the spiral form which is the etiological agent in Asiatic cholera. This species Koch likened to the German "comma," calling it the "comma bacillus." These curved organisms multiply by transverse fission, the curves of the new cells being turned in a direction opposite to the original. When they remain attached to each other a chain of vibrios results and looks like a true spiral. At the present time a rigid cylindrical organism slightly bent or twisted on itself but not forming a complete spiral is called a "vibrio."

D. *Spirillum.*—Rigid organisms bent or curved on themselves in such a way that one end of the organism always comes back to a point in the same plane as the other end are called "spirilla." The term "spirillum" is often used synonymously with "vibrio" to indicate any rigid curved organism regardless of whether a true spiral is formed or not.

E. *Spirocheta.*—The term "spirochæta" was introduced by Ehrenberg for spiral organism with a flexuous body capable of twisting or bending in various directions. The species described by Ehrenberg is a free-living form, but the term "spirochæta" is now utilized for a large group of motile spiral organisms, some of which are highly virulent for man and the animals. The spirochetes differ from the spirilla in that the body of the spirillum is rigid, that of the spirochæta flexuous. The motility of the spirillum is determined by its flagella, that of the spirochæta apparently by an inherent contractibility of the body cell.

F. *Leptotrichia.*—The term "leptotrichia" was introduced by Trevisan for a filamentous cylindrical organism looking like a long bacillus or bacterium, and this significance is still retained. The leptotrichias are often slightly bent or curved, but never form a true spiral or exhibit inherent motility. These forms are usually described as leptothrix.

G. *Actinomyces.*—A large number of organisms have been described which differ from those already mentioned in their mode of reproduction. They grow in threads and show true branching, new cells arising by a branching of the original threads. In addition, they go through a complex cycle of development which allies them with the lower plants. They are often spoken of as the "higher bacteria." The term "streptothrix" has also been used for some of the species described now as "actinomyces." The exact significance of the term "streptothrix" is in some doubt and at the present time the term "actinomyces" is employed for all the filamentous bacteria in which true branching and spore-formation occur.

H. *Cladothrix.*—Organisms growing inside a sheath or capsule and multiplying by transverse fission inside this capsule are called "cladothrix." When the sheath becomes crowded with cells it ruptures and a new sheath is formed about a portion of the newly formed cells. This phenomenon is called false branching because there is no true branching of the original bacteria.

Other terms employed for some of the "higher bacteria" are "crenothrix," in which the cells divide transversely, and then in all three directions of space, spherical organisms being eventually produced; "phragmidiothrix," in which branching forms develop inside delicate sheaths; "thiothrix," in which unbranched cells containing sulphur grains are enclosed in a delicate sheath; and "beggiotoa," thread-like organisms not enclosed in sheaths, the cells containing sulphur granules. Finally, there are many terms in common use among bacteriologists which have an exact significance, but which are not adopted by the systematists. The name "streptobacillus," for instance, is given by many bacteriologists to bacilli growing in chains and is thus analogous to the streptococcus, signifying a coccus growing in chains.

## THE SIZE OF THE BACTERIA

The various living organisms, now grouped together as the bacteria, differ markedly in size, but no more than do the members of other groups of plants or animals. In early investigations their size was determined by comparing them with the red blood-corpuscles of animals or man. At the present time their size is expressed with a micron or $\frac{1}{1000}$ of a millimeter as the unit, equivalent to about $\frac{1}{25000}$ of an inch and indicated by the Greek letter $\mu$. A ruled eyepiece micrometer is put in the ocular of the microscope and the size of its divisions determined by comparison with a ruled stage micrometer of a standard length. This gives the value of each line and division of the eyepiece micrometer for each combination of ocular and objective and represents a constant unit of size. The measurements obtained by this method are at first apt to be regarded as inaccurate. It may be noted that individual members of the same bacterial species vary greatly according to their age and the medium in which they have been living. In consequence, these measurements, which are only approximate at best, give us a very good idea of their relative size. A more accurate method of obtaining the size of the bacteria is to project them on a screen by means of photographic lenses giving a constant magnification. The size of the image found on the screen can be measured exactly and the real size of the micro-organisms found by dividing the dimensions of the image by the magnification of the lenses. Finally, the size of the bacteria may be gotten by the use of a filar micrometer eyepiece provided with a micrometer disk and a calibrated screw. As the micrometer disk is moved from one part of the organism to another the number of divisions through which the screw turns is read. Since each turn of the screw moves the disk a certain definite distance, the space through which the point of the disk is moved can be determined with considerable accuracy. While this method of obtaining the size of the bacteria is probably the most reliable, it is unfortunately only applicable to the larger forms.

The size of the bacteria varies greatly among the individuals of the same species and depends upon a number of circumstances. Young actively growing organisms are usually larger than older individuals, as has been shown by Clark. They are larger when growing in fluid media than when

developing on a solid substratum. When the reaction of the medium is alkaline they are usually smaller than when it is acid. Acidity in general increases the size of the majority of organisms. There are also differences in size among different strains of the same species. Some strains of the pyogenic cocci, like *Staphylococcus pyogenes aureus*, are distinctly larger than other strains and yet both may have the same biological properties and the same pathogenic action. Similar differences may occasionally be noted among different strains of the intestinal bacteria as, for example, with *Bacterium coli commune*. Finally many organisms produce large forms in the tissues or on special media. The streptococci may appear larger in infected animals than under artificial conditions. The plague bacillus, *Bacillus pestis*, produces large forms in the tissues and very large forms when grown on salt agar.

Among the larger bacteria may be mentioned *Bacillus Bütschlii* of Schaudinn and *Bacillus buccalis maximus* of Miller. Such species lend themselves to exact morphological studies because of their size. Thus the elements of *Bacillus Bütschlii* may be 4 to 5 $\mu$ wide and 50 to 80 $\mu$ long, while *Bacillus buccalis maximus* varies in length from 8 to 27 $\mu$ and in width from 1.4 to 1.9 $\mu$.

Among the smaller organisms we have such species as the *Micrococcus minutissimus* and the *Micrococcus Mesnili* of Borrel. Such organisms are about 0.2 $\mu$ in diameter.

Below this size micro-organisms become difficult to measure and appear as minute points or dots in the microscopic field. Whether there are bacteria beyond the powers of our present microscopes to reveal is still a mooted question, but the existence of the so-called "viruses," materials which will produce disease in plants and animals, but in which no organisms are visible, leaves no doubt that infectious matter of some form or other can exist which cannot be seen with even the high-powered microscopes of the present day.

The majority of the bacteria and particularly the pathogenic varieties occupy an intermediate position between such extremes as may be noted with the very large and the very small organisms. The staphylococci, for instance, are about 0.5 to 0.6 $\mu$ in diameter, the bacteria like the typhoid bacillus and the dysentery bacillus about 0.5 $\mu$ in width and 2 to 3 $\mu$ in length. The spore-bearing organisms, such as *Bacillus anthracis* and *Bacillus Welchii*, are usually somewhat larger. *Bacillus anthracis*, for example, measures 0.75 to 1 $\mu$ in diameter and 4 to 6 $\mu$ in length. Occasionally very large spore-bearing organisms are encountered like *Bacillus megatherium*, the average width of which is over 1 $\mu$ and the average length 8 to 10 $\mu$.

Finally, the diameter of organisms or the width, as it is often termed, is far more constant than the length. As organisms grow they increase greatly in length, their width remaining fairly constant. This phenomenon is associated with their multiplication by transverse division.

## THE WEIGHT OF THE BACTERIA

According to Rubner[1] the specific gravity of the common bacteria varies from 1.038 to 1.065. Stigel[2] has found that the specific gravity varies with the medium, ranging from 1.120 to 1.350, older cultures having a lower specific gravity than the young of the same species. Kendall[3] has recently suggested an ingenious method of obtaining the weight of a single organism in the species *Bacillus coli*. These organisms measure about 1 $\mu$ in width by 2 $\mu$ in length and have the shape of an elongated cylinder. The volume

of a cylinder equals the product of the square of the diameter and the factor 0.7854, multiplied by the length of the cylinder. The volume of a single colon bacillus would thus be $(0.001)^2 \times 0.7854 \times 0.002 = 0.00000000157$ cm. The weight equals the volume multiplied by the specific gravity. The specific gravity of *Bacillus coli* may be taken as 1.040. Each *Bacillus coli* weighs therefore 0.00000000613 mg. Each human red blood-corpuscle weighs about 0.00008 mg. or about 50,000 times the weight of each individual cell of *Bacillus coli.*

## THE CELL WALL

The cell wall enclosing the bacterial cytoplasm is a delicate membrane which appears sometimes as a faint line in unstained material. It has the same affinity for the anilin dyes as the cytoplasm and in consequence is not to be differentiated from it in stained material. At times in old faded preparations or in films treated with decolorizing agents one can get the impression of a distinct membrane. It is easily demonstrated by the phenomenon of *plasmolysis* described by Fischer. If the bacteria are put into solutions of salts of a concentration greater than the salt concentration within the cells, *i. e.*, hypertonic solutions, the cell fluid runs out leaving vacuoles and masses of cytoplasm just inside the intact walls. Much the same thing is shown in old cultures when the cytoplasm has disintegrated and passed out of the cell, leaving the empty membranes, the so-called "shadows." Portions of fragmented cell walls are also occasionally visible when the bacteria are put in solutions of a lesser tonicity than is normal to them, that is, hypotonic solutions. Here the increase of pressure resulting from the absorption of water bursts the bacterial walls and the contents are rapidly dispersed. This is known as plasmoptysis. Not infrequently parts of the cell wall remain behind for brief periods.

In many bacteria the cell walls give a blue color with chloriodid of zinc. This is sometimes described as the cellulose or hemicellulose reaction and is one of the characteristics of the bacteria which affiliates them with the higher plants. Dreyfuss[4] has found cellulose in the pyogenic bacteria and in *Bacillus subtilis*, Hammerschlag claims to have obtained cellulose from tubercle bacilli, Dzierzgowski and Rekowski[5] have found it in the diphtheria bacillus and Tamura[6] has demonstrated hemicellulose in the same organisms.

In other instances, as with the species known as *Bacterium xylinum*, Emmerling[7] has found a substance like chitin, the material characteristic of the outer integument of the crustacea and the insects. Iwanoff[8] has made a chemical analysis of the cell membranes of *Bacillus pyocyaneus*, *Bacillus megatherium*, and *Bacillus anthracis*, finding 46 per cent. of carbon, 6.7 to 7 per cent. of hydrogen, and 8.4 to 8.8 per cent. of nitrogen. This is much like the analysis given for chitin which is a polymer of glucosamin, $CH_2OH(CHOH)_3CHNH_2CHO$. Such a composition would affiliate the bacteria with animal rather than with plant forms.

The cell membrane of the higher bacteria may contain iron salts (Welch).

## THE BACTERIAL CYTOPLASM

Just inside the cell wall lies the bacterial cytoplasm, the outer part of which is sometimes spoken of as the ectoplasm. When the bacteria are examined unstained it is seen to be very finely granular at times, smooth and homogeneous as a rule. With some of the larger bacteria the granules may be very abundant. Our information about the cytoplasm is still limited and consists chiefly in the demonstration of particular substances

and particulate matter in it, the amount of which varies with the different species. The most significant of these are the following:

1. Metachromatic granules described by Babes[9] and by Ernst[10] and also called the "Babes-Ernst bodies." These are often arranged at the poles of the bacteria and are then called "polar granules" or "polar bodies." These granules take up the basic anilin dyes with avidity and stain well with methylene-blue or Bismarck brown. They are very common in the larger bacteria, as *Bacillus oxalaticus* or *Bacillus megatherium*, and are also present as minute dots or points in certain sarcinas and in the gonococcus. In some forms metachromatic granules are highly developed, as in the diphtheria bacillus, and their presence and arrangement are often an important guide in the identification of species.

2. Chromatin-like bodies described by Arthur Meyer[11] as volutin. They stain blue by methylene-blue containing 1 per cent. sulphuric acid, deep red by carbol fuchsin containing 1 per cent. sulphuric acid, the cytoplasm being decolorized. They are Gram-negative. The volutin of Meyer is probably the same as the metachromatic granules of Babes and Ernst. It is present in great abundance in certain species of spirilla, as in *Spirillum volutans*.

3. Glycogen, staining brown with a dilute solution of iodin containing potassium iodid. This is present in some of the larger bacteria of the mouth, especially when they are growing in solutions containing carbohydrates. It may also be found in the tubercle bacillus (Levene[12]).

4. Fat particles, staining black with 1 per cent. solution of osmic acid. These are present in some of the larger bacteria, particularly *Bacillus oxalaticus* or *Bacillus mesentericus*. Fat is also present in *Bacillus tuberculosis*.

5. Acid-fast particles. Bodies staining with difficulty with the anilin dyes, requiring long exposure and frequently gentle heating and subsequently resisting the decolorizing action of dilute acids and alcohols, are known as acid-fast particles. They are present in a considerable number of species which are consequently described as the acid-fast bacteria. This group includes such important pathogens as the tubercle bacillus and the leprosy bacillus. The composition of the acid-fast particles is not known with certainty. It has been regarded as a waxy substance or a higher alcohol (Bulloch and MacLeod[13]).

6. Sulphur granules. These are present in some of the higher bacteria like the beggiotoa and in certain species known as the sulphur bacteria. They are found when these species are developing in water containing $H_2S$. Sulphur granules may be dissolved out of the organisms by carbon bisulphid and deposit as characteristic crystals.

## NUCLEAR MATERIAL

The earlier students of bacteriology made an exhaustive search for a morphological nucleus in the bacteria, but no such structure was demonstrable. This led to the prevalent view that the bacteria are non-nucleated organisms. They exhibit a special affinity for nuclear dyes and later workers came to the conclusion that the bacterial cell in its entirety represents the nucleus of higher structures. Improvement in cytological technic and more careful study of staining reactions have shown that nuclear material can be demonstrated in practically all bacteria. This material is arranged as a kind of network running through the cell and is described as diffuse nucleation. At times morphological nuclei have been stained in forms

regarded as bacteria, but more thorough study has led to the conclusion that these organisms are probably not true bacteria, but represent some other types of plant life. An interesting exception to the general rule that bacteria have no morphological nuclei has been noted by Dobell[14] who has described such a structure in his peculiar form, *Paraspirillum Zedrowski.* Dolene also has found well-defined chromatin threads in some of the larger bacteria and nuclear material in a number of smaller sarcinas and cocci. Zetnow[15] has found nuclear material in large organisms like *Bacillus megatherium.* According to Park, Williams, and Krumwiede[16] small masses of nuclear material can be stained in a number of organisms like *Bacillus anthracis.*

The view now held by the majority of bacteriologists is that the bacterial cell represents a cytode in Haeckel's sense or a plasson of Van Beneden, containing finely mixed nuclear material (chromatin) and cytoplasm (plastin). The relative amounts of these substances in the bacteria correspond to the nuclei of higher plants rather than to their cytoplasm.

## ORGANS OF LOCOMOTION

Many bacteria are devoid of inherent ability to move from one place to another, their change of location being caused by currents in the fluids surrounding them. Others are possessed of independent motility and move rapidly from place to place. This ability to move depends upon the presence of cilia or flagella in the bacteria and upon the presence of contractile elements in the cytoplasm in the flexuous spirochetes. These flagella or cilia are delicate hair-like appendages attached to the membrane of the bacteria and are thought to be prolongations of the cytoplasm through the cell wall. They are extremely fragile and are washed off by the ordinary procedures for fixing and staining. They may be seen under dark field illumination as fine lashing structures which refract the light differently from the bacterial bodies. Flagella were first observed on some of the larger bacteria and probably first definitely demonstrated by Koch on photographic plates. Koch subsequently devised a method for staining them, and such methods were perfected by Loeffler, van Ermengem, Pitfield, and many others. Cilia are so fragile that they are difficult to demonstrate by the best technic, and considerable difference of opinion still exists as to the flagellation of many of our most common species.

The flagella or cilia are endowed with inherent motility due probably to contractile elements in their substance. They vary greatly in length, the shorter being several times as long as the body of the bacillus, the longer many multiples of it. They are attached either to the ends or to the sides of the organisms. When attached to the ends they are either single or consist of a closely twined or twisted bunch of four, five, or even more. When attached to the sides they are more numerous, varying from sixteen to twenty-four in number. They may project out from the organism in a straight line or be coiled up about the organism as Moore has pointed out for the hog-cholera bacillus. The motility of the organism varies somewhat with the point of attachment. When the flagella are situated at the poles their lashing vibration gives the organism a rapid whirling or whizzing motion. If a large number of organisms happen to be in the field at the same time the whole mass is seen to be in violent agitation. This was likened originally by Koch in the cholera vibrio to the swarming of gnats. When the flagella are attached to the sides they drive the organisms forward or backward in a kind of swimming movement which may be very rapid or at times somewhat

leisurely. The vigor of movement of the flagella varies greatly with the age of the organisms. Young cultures are in general more actively motile than old cultures and are on that account usually selected for the demonstration of cilia. In the spore-bearing organisms the new vegetative rods which have just emerged from the spores are actively motile, the flagella having been developed within the first few hours of vegetative life. This period in the life-history of these organisms has been called the "swarming stage" by Arthur Meyer and Chester.

Little is known of the chemical composition of the flagella. It must differ from that of the cytoplasm since Theobald Smith and Reagh and Orcutt have shown that it calls forth different agglutinins on introduction into animals. Gotschlich[17] states that the changes in bacterial flagella in plasmolysis indicate that they have a lower water content and are more concentrated than the bacterial cytoplasm.

## CAPSULES

Certain species of bacteria are surrounded by a peculiar mucinous capsule which gives them a viscid character when in masses. These capsules are occasionally visible in unstained material, but are washed off in the usual methods of fixing and staining. They may be stained after the use of reagents like dilute acetic acid which precipitate the capsular substance and then appear as more or less distinct masses of material entirely surrounding the organism. Certain species are characterized by the presence of a capsule, as the pneumococcus, in which each pair of organisms is contained within a rather small delicate mass of capsular material the outer edge of which often appears as a faint rim. In other species, as *Bacillus lactis aërogenes* or the Friedländer bacillus of pneumonia, the capsular substance is more abundant and may surround two or three organisms at the same time. Such species as these, and a number of others like *Bacillus Welchii*, are always provided with capsules which serve as a valuable means of iden ification and are present both in cultures and in the tissues of inoculated animals. In other species, such as *Bacillus anthracis*, they are found only in the animal body, not in artificial cultures. In these cases the capsule may serve as a covering which protects the organism against the destructive agencies of the animal body. A similar action may be attributed to the capsules of the pneumococcus or the Friedländer bacillus. Under artificial conditions of cultivation capsules often fail to develop and may be extremely difficult to find. In addition to these capsules, which are to be regarded as an integral part of the species under normal circumstances, capsules are often formed or deposited on bacteria under certain conditions of growth. Thus the ordinary non-pathogenic spore-bearing bacteria like *Bacillus subtilis* or *Bacillus mesentericus* may become encapsulated when growing in the intestinal contents, in bread dough, and in similar materials. Such structures are rapidly lost on artificial cultivation, disappearing after one or two generations. Finally certain species not ordinarily encapsulated may form viscid material like that of capsules on artificial media under conditions which are not entirely understood. Thus Theobald Smith[18] has reported that growths of the tubercle bacillus may be viscid after repeated transfer and Weleminsky[19] has observed a similar phenomenon in the glanders bacillus and in the organisms producing septicemia in rabbits. There is some evidence that capsules are of two kinds, the true capsules produced by the organism as a kind of secretion, and culture capsules forming in the culture-media and deposited on the outside of the organism.

Various attempts have been made to learn the chemical composition of the capsular substance. It is sometimes called a mucin, again a pseudomucin and sometimes a glycoprotein. When heated with acids it gives a reaction with Fehling's solution and evidently contains a carbohydrate radicle.

## MULTIPLICATION

Bacteria multiply by simple or binary fission, each organism dividing into two daughter cells. This may be demonstrated by actual observation of living specimens. A delicate constriction appears in the center of the organism and gradually deepens until one cell is nipped off as it were from the other cell. The new-formed cells are approximately of equal size. The entire process takes place in a short time, sometimes in fifteen minutes. Many attempts have been made to show some rearrangement of the nuclear material in the dividing cells, but thus far nothing comparable with the division of the nucleus has been made out with certainty. Nakanishi[20] has observed that in the diphtheria bacillus the transverse septum appears at the site of a metachromatic granule, but no division of these granules has been shown to occur. In certain species like the diphtheria bacillus, as transverse division takes place, the daughter cells seem to spring apart suddenly by a kind of snapping motion, the new-formed cells lying at an acute angle with each other. The elongated bacteria never divide by longitudinal fission. If careful measurements are made cells twice as long as the shortest forms are frequently seen, never cells twice as wide as the narrowest forms. The former phenomenon is consistent with transverse division. The failure of the latter phenomenon rules out longitudinal division.

Certain types of bacteria multiply by true dichotomous branching and these organisms have usually been set apart from the simpler bacteria like the cocci and bacilli and spoken of as the "higher bacteria." There is some confusion in terminology and the species showing true branching have at times been called streptothrix, at other times actinomyces. The actinomyces produce spore-like bodies or conidia and have a characteristic arrangement in rosettes in the animal body, and it seems probable that this name has priority over streptothrix. The true branching characteristic of these forms is to be distinguished from the false branching of the cladothrix in which the cells multiply within a sheath which itself ruptures or branches when it becomes too crowded with cells. Occasionally other organisms like the tubercle bacillus or the diphtheria bacillus show true dichotomous branching, in consequence of which these organisms are also separated from the simpler bacteria. In addition, certain species of organisms found in the intestines, especially *Bacillus bifidus* of Tissier, is so regularly branched that this phenomenon must be regarded as characteristic of it. With this species it is not certain the branching signifies actual multiplication. Finally, branched or bifurcated forms are rarely encountered on ordinary bacteria like *Bacillus coli*. Such appearances are probably to be interpreted as involution.

## SPORE FORMATION

Resistant bodies, now known as endogenous or true spores, were originally observed by Pasteur in decomposing fluids and called by him "corpes brillantes." They were subsequently described by Cohn in *Bacillus subtilis* and by Koch in *Bacillus anthracis*. Koch worked out the complete life cycle from vegetative rod to spore and back again to vegetative rod. Since then spores have been described in a large number of aërobic and anaërobic species. They occur chiefly among the rod-shaped cylindrical bac-

teria, rarely among the spirilla, never among the micrococci. Spores have been described among the spirochetes, but their presence has not been confirmed. Within the past few years especial attention has been devoted to spore formation by Arthur Meyer in Germany and by Chester in America.

Spores are formed inside the body of the organisms and first appear as minute masses of material more highly refractile than the cytoplasm of the cells which frequently increase in size just before the spores begin to be produced. As these small refractile bodies enlarge they take up a position often characteristic for the various species of organisms. They may remain in the center of the rods and are then spoken of as central spores. Or they may migrate toward one end or the other, coming to occupy a subterminal position just beneath the end. Finally they may actually pass out of the cell and appear on the very tip of the organism. These are the true terminal spores and are peculiar to certain species such as the tetanus bacillus. During their migration the spores enlarge and the degree of enlargement varies again with the different species. Some spores remain small, less than the diameter of the organism producing them. The majority, however, grow to considerable size and are usually larger than this diameter. The organism producing the spore and still containing it is called the sporangium. A form which enlarges at sporulation is called a clostridium or plectridium. As the spore matures the cytoplasm of the rods disintegrates and gradually disappears. Sometimes a portion remains attached to the spore for a time as a kind of sheath or cap. Eventually this also disappears, leaving the mature spore consisting of a resistant membrane and the spore contents. The spores vary greatly in shape and are round, oval, or cylindrical. If these mature spores are put under proper conditions of moisture and heat they in their turn produce vegetative rods. They enlarge somewhat, the spore membrane disintegrates at some point, and the new rod pushes its way out of the spore. As the rod develops it leaves the spore membrane behind and this is eventually cast off. The vegetative rods may emerge from the ends of the spores, this process being spoken of as polar germination. Or they may emerge from the middle and the germination is then called equatorial. The complete cycle of changes consists in the formation of sporangium, spore, and vegetative rod. With many of the spore-bearing organisms the young vegetative rods are highly motile.

Spores are difficult to stain and remain colorless by ordinary methods of fixing and dyeing, being recognized by their high refractility. Occasionally the masses of cytoplasm left attached to the spores may be stained and various special methods have been devised to stain the spore itself. These methods depend for their success upon the use of heat and prolonged exposure to the dye as a result of which the spore wall is finally penetrated by the stain. Once stained, the spores resist decolorization. The spore membrane is thought by some authors, notably Meyer, to consist of an outer exine and an inner intine.

True spores are characterized by their great resistance to heat and to the action of disinfectants. While ordinary vegetative bacteria are destroyed by exposure to temperatures ranging from 55° to 65° C. for brief periods of time, one-half to one hour, spores withstand such temperatures and are only destroyed at temperatures ranging from 85° to 100° C. Indeed, some types of spores have been described which resist the heat of boiling water, 100° C., for as long as twenty-four hours. Spores are readily killed by heat under pressure when the temperature reaches a few degrees above the boiling-point of water. They withstand the action of dilute acids and alkalis and are destroyed only after considerable time. They withstand drying for

long periods, months and even years. Thus the spores of the anthrax bacillus have been shown to be viable after twelve to fifteen years.

The function of spores in the life-history of the organisms producing them is not entirely clear. They are not a sign of multiplication, since but a single spore is formed in each rod and a single vegetative form from each spore. Instances of multiple spore formation and germination have been described, but never been definitely confirmed. Whatever their purpose, spores represent resistant stages through which organisms pass in their development. They are sometimes regarded as resting stages, the so-called "Dauerformen" of the Germans.

Conditions which make for the formation of spores differ with the organisms. In some instances, as with *Bacillus anthracis* and *Bacillus subtilis*, spores are formed when the conditions of growth seem to be at their optimum. They are more apt to develop in alkaline than in acid nutriment. With some species, as with *Bacillus anthracis* and *Bacillus Welchii*, they are never formed in infected tissues. In other instances, as with *Bacillus tetani*, they are produced abundantly in such areas. They sometimes form in the presence and sometimes in the absence of oxygen. It has been suggested that the spore appears under unfavorable conditions and is for the purpose of ensuring the continuity of the species. There is little experimental evidence in favor of this view.

Reinke[21] believes that the protoplasm of the spores may be an anhydrid of the vegetative cell cytoplasm. Their chemical composition is little understood. Cramer[22] has made an analysis of the spores of *Penicillium glaucum*, closely related to the bacteria, with the following results:

| | Per cent. |
|---|---|
| Protein | 28.44 |
| Ether extract | 7.30 |
| Alcohol extract | 30.40 |
| Cellulose | 11.10 |
| Starch | 17.00 |
| Ash | 1.90 |
| | 96.2 |
| Undetermined portion | 3.8 |

Such spores have thus a chemical composition closely similar to that of the seeds of higher plants.

## INVOLUTION FORMS

In old cultures and in cultures developing under unfavorable circumstances a considerable change in the morphology of the bacteria is noticeable. Instead of the ordinary regular forms taking the stain homogeneously, various enlarged distorted poorly staining elements appear. Such organisms were called arthrospores by Hueppe, but they have none of the resistance to heat and disinfectants of true spores. They are rather to be regarded as either a sign of degeneration of the organism or a change in the morphology due to environmental influences. Such involution forms are produced by practically all bacteria from time to time, but they are especially marked in certain species. Old cultures of streptococci for instance occasionally show distorted bacillary forms quite different from the characteristic cocci. In the vibrio of Asiatic cholera cultures two to three days old often exhibit an abundance of large poorly staining sickle-shaped elements which can hardly be recognized as vibrios, or a mass of rather irreg-

ular indistinct granules. Transfers from such degenerated cultures are often successful, the organisms reverting to their normal size and shape when restored to favorable media. In the plague bacillus, *Bacillus pestis*, the formation of large poorly staining coccoid bodies is common in cultures and similar but smaller bodies occur in the tissues of infected animals. These involution forms in the plague bacillus are characteristic and a considerable aid to identification. They develop especially on agar containing a high concentration of salt. Swollen distorted forms occur with many of our common species, such as *Bacillus coli*, when the cultures are grown on acid media or on sugar media which turn acid from the fermentation of the carbohydrates. Such changes in morphology on acid media may be due to a change in permeability of the cell membrane from the acid. This results in a greater absorption of water in consequence of which the cells become swollen and distorted. The large irregular forms of bacteria described as involution or degeneration may also be considered as "teratological" forms.

## THE CHEMICAL COMPOSITION OF THE BACTERIA

Many attempts have been made to learn something about bacterial activity by chemical analysis of the bodies of the organisms themselves. The first chemical analysis of the bacteria was made by Nencki and Schaffer[23] who examined the putrefactive bacteria and found them to have the following percentage composition:

| | |
|---|---|
| Water | 83.42 |
| Protein | 13.96 |
| Extractives | 1.00 |
| Ash | 0.78 |
| Residue | 0.84 |
| | 100.00 |

Similar results have been obtained by Koppes[24] in *Bacillus prodigiosus*, the analysis of which results as follows:

| | |
|---|---|
| Water | 85.45 |
| Protein | 10.33 |
| Extractives | 0.70 |
| Ash | 1.75 |
| Residue | 1.77 |
| | 100.00 |

Ruppel[25] also analyzed *Bacillus tuberculosis*, with the following results:

| | |
|---|---|
| Water | 85.00 |
| Protein | 8.50 |
| Extractives | 4.00 |
| Ash | 1.40 |
| Residue | 1.10 |
| | 100.00 |

Such analyses give us a crude idea of the kind of chemical structure possessed by the bacteria. They are seen to consist largely of water and protein materials. The ash and extractives form but a small part. This large percentage of water is probably the rule for all organisms. Thus Nicolle and Alilaire[26] have analyzed a number of pathogenic organisms with the same result. The bacteria indeed may be looked upon as "water plants."

In certain species such as the tubercle bacillus various extractives are more abundant than in ordinary bacteria. Thus in Ruppel's analysis the extractives amounted to 4 per cent., this being due to the presence of a waxy substance which gives this organism its peculiar acid-fast staining reaction. This substance was regarded by Ruppel as a true wax. Later investigations by Bullock suggest that it may be a higher alcohol allied chemically to glycerin.

Important as such chemical analyses of the bacterial cells may be, they give us little real knowledge of the chemical groups which may be present within them. More valuable knowledge has been obtained by a study of the protein and nuclear material which may make up anywhere from 30 to 80 per cent. of the dried bacteria. Thus Ruppel has made an analysis of 100 grams of dried tubercle bacilli, with the following results:

| | Grams. |
|---|---|
| Nucleic acid (tuberculinic acid) | 8.50 |
| Nucleoprotamin | 24.50 |
| Nucleoprotein | 23.00 |
| Albuminoids, keratin, etc. | 8.30 |
| Fats and wax | 26.50 |
| Ash | 9.20 |
| | 100.00 |

In addition to investigations of this character, observations have been made upon the isolation and identification of various individual chemical groups in the bacterial cells. Thus nucleins and nucleoproteins have been found in *Bacillus subtilis* by Vandevelde,[27] in *Bacillus pestis* by Lustig and Galeotti,[28] in *Bacillus typhosus* by Paladino-Blandini,[29] and in *Bacillus diphtheriæ* by Aronson.[30] Glyconucleoprotein has been obtained by Carapelle[31] in *Bacillus prodigiosus*. Xanthin has been found in plague bacilli by Lustig and Galeotti and xanthin bases have been observed in the dried residue of a water bacillus by Nishimura.[32] Glyconucleoproteins have been found by Vaughan[33] in *Bacillus coli*.

## PIGMENT PRODUCTION

Bacteria produce pigments which range in color from green and blue to orange and red. Some of these pigments diffuse out of the bacterial cell while the organisms are still intact and color the surrounding medium. The species forming pigments of this character are called *fluorescent* by bacteriologists. They are common in water (*Bacillus fluorescens liquefaciens* and *Bacillus fluorescens non-liquefaciens*) and the pigments arising from their growth are usually green or greenish yellow, although blue diffusible pigments have been occasionally described. The pigment formed by the so-called fluorescent bacteria is water soluble and appears yellow if viewed by transmitted light and green by reflected light. It is called bacteriofluorescin by Lehmann. It is insoluble in alcohol, ether, and carbon-bisulphid. Aqueous solutions are blue in neutral reactions, green in alkaline, and not fluorescent when the reaction is acid. Strictly speaking the term "fluorescence" should be applied to substances which emit light only when light is being absorbed. Light of one wave length falls on a substance and results in emission of light of another wave length.

In other cases the pigments which are manufactured by bacteria do not diffuse out of the bacterial cell during life, but are liberated when the cells die and disintegrate. The color which arises from the growth of these

organisms depends upon the presence of the bacteria themselves except in old cultures where a number of cells have broken down and their pigments have been liberated. The bacteria which produce these non-diffusible pigments are called *chromogenic* by bacteriologists. The coloring-matter is insoluble in water, but soluble in alcohol, ether, or carbon bisulphid. *Bacillus prodigiosus* and its varieties, *Staphylococcus aureus* and *Sarcina lutea*, are examples of chromogenic bacteria.

The capacity of pigment production varies in intensity with different strains of the same species and is influenced by a number of factors. Pigments are usually formed only at low temperatures under aërobic conditions with free access of atmospheric oxygen. Rarely they are produced under anaërobic conditions, as with *Spirillum tyrogenum* of Denecke and with the acne bacillus which elaborates reddish pigments only in the absence of oxygen. In addition, colorless water-soluble substances (leucobases) are produced by bacteria under anaërobic conditions and these immediately develop into the characteristic pigments on exposure to air. This is true of *Bacillus pyocyaneus*. Certain media like potato enhance color production. Freshly isolated organisms often produce pigment in abundance and lose this property after cultivation on artificial media. Under such conditions the characteristic may occasionally be restored by cultivation at low temperatures on favorable media. Finally the phenomenon of color production may occasionally be seen in organisms which do not normally possess this power. Thus the diphtheria bacillus rarely produces an orange-yellow pigment and the tubercle bacillus a yellow or reddish pigment.

Pigments vary greatly in their chemical behavior with solvents. Some are soluble in chloroform, alcohol, ether, and petroleum, others soluble in only some of these substances. They differ markedly in their oxidation products and from some of them crystalline compounds of unknown chemical composition have been isolated (Fischer[34]). The sulphur bacteria produce a special type of pigment known as bacterio-purpurin, characterized by its capacity to absorb the rays of light in such a way that the organisms build up carbohydrates and other organic compounds from carbon dioxid and water. This property is spoken of as photodynamic. Bacterio-purpurin resembles somewhat the chlorophyl of the higher plants (Molisch[35]).

Zopf[36] regards the pigments produced by bacteria as belonging to the lipochromes which occur widely in plants and animals. The coloring-matter of animal fat and the coloring-matter of the petals of the ranunculaceæ are lipochromes, as well as the yellow pigments in blood-serum and the yellow substance in the yolk of eggs. Beijerinck divides the bacteria producing pigments into two classes, the chromophorous in which the pigment is inside the cells and the cytoplasm saturated with it, and the chromoporous which distribute the pigment outside the cells. Thus the purpur-bacteria are chromophorous and in them pigment production is related to vital activity. The majority of bacteria are chromophorous and their pigment is apparently of no value.

Finally dark brown coloring-matter is produced by several species of bacteria like *Bacillus coli* and *Bacillus typhosus*. This material arises by oxidation from tyrosin through the agency of an enzyme, tyrosinase.

## LUMINESCENCE

Several species of micro-organisms produce substances which are luminescent in the dark. These organisms were formerly called phosphorescent, but are properly designated as luminescent. Such organisms occur chiefly

in sea-water and are easily cultivated on ordinary media to which salt is added, usually 2 to 3 per cent. Magnesium chlorid ($MgCl_2$) gives better results than sodium chlorid (NaCl). The light is greenish or bluish and so powerful that one can read the hands of a watch by the luminescence of a gelatin stab. Lode[37] has estimated the light given off from a vibrio isolated from the water of the river Elba as $785.10^{-10}$ candle-power for its square centimeter (1270 qm. necessary for light of 1 candle). Friedberger and Dopter have obtained higher values for *Bacterium phosphorescens*. The development of luminescence is bound up with the vital activities of the bacterial cells and a free supply of oxygen is necessary. The substance producing it has not thus far been isolated. Clark[38] states that this type of luminescence is a chemiluminescence as distinguished from photoluminescence which includes fluorescence and phosphorescence.

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## Chapter III

# BACTERIOSCOPIC METHODS

### MICROSCOPIC EXAMINATION

**The Microscope.**—For modern bacteriological work high-powered microscopes are essential. With those giving a magnification of 500 to 600 diameters larger organisms like the anthrax bacillus, certain species of leptothrix and actinomyces, and some of the larger non-pathogenic spirochetes are brought into view. With a magnification of 1000 diameters the majority of pathogenic bacteria and the non-pathogenic bacteria of importance are clearly visible. They are better viewed with a magnification of 1250 to 1280 diameters afforded by the improved microscopes of the present day. Microscopes giving a magnification higher than this are not of great value in bacteriology, since the lack of illumination interferes materially with the clearness of the picture obtained. Rarely a magnification of 1500 diameters, which may be gotten by the use of apochromatic objectives and

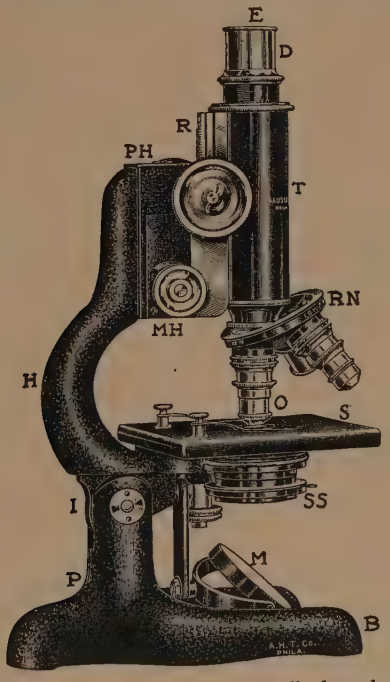


Fig. 1.—A satisfactory type of microscope for medical work: E, Eyepiece; D, drawtube; T, bodytube; RN, revolving nosepiece; O, objective; R, rack; PH, pinion head for coarse focusing; MH, micrometer head for fine focusing; H, handle; S, stage; SS, substage; M, mirror; I, inclination point; P, pillar; B, base.

oculars, is a help in making out some of the details of morphological structure. Occasionally minute particles which are not visible with a lens of 1200 to 1300 diameters may be brought into view, but in general these

appear as minute points which are recognized as micro-organisms only with great difficulty.

The illustration shown on page 47 gives the details of a modern high-powered microscope.

*Dark Field.*—In illumination by the dark field the light is introduced into the microscopic field at an angle instead of directly as in ordinary bacteriological work. Certain objects are brought into view by virtue of their refractility. This method is not available for stained preparations. It is especially valuable for the study of spirochetes and for small objects which resist staining and are visible by self-illumination.

**Examination of Living Material.**—*The Hanging Drop.*—Micro-organisms in the living condition may be examined by placing a small drop of material between an ordinary slide and coverslip, the space between sufficing for the movements of active forms. A better method is to place the material on a thin coverslip, drop this on a hollow ground slide, and seal the edges with vaselin or oil, the so-called hanging drop. For the determination of motility it is essential that the bacteria be placed in some isotonic fluid like physiological salt solution, blood-serum, broth, or peptone solution. In distilled water the organisms are plasmolyzed rapidly. Ordinary tap-water usually contains a sufficient amount of salts to render it isotonic and suffices for the determination of motility. Young cultures from six to twenty-four hours old are better adapted for this purpose than older cultures. Motility is sometimes best seen in broth or fluid serum cultures. Sometimes young agar cultures six to seven hours old show actively motile organisms. This is especially true of spore-forming bacteria in which motility is apt to cease with the formation of the spores. For the determination of motility the high dry lens frequently suffices. The oil-immersion lens may be employed.

*The Hanging Block.*—In order to study the development of bacteria Hill has devised an apparatus known as the hanging block. This consists of a small square piece of agar cut from an agar plate about 2 to 3 mm. in thickness and about the size of an ordinary coverslip. This is dropped on a slide, the surface inoculated with the bacteria under investigation, covered with a coverslip, and examined with a high dry or an oil-immersion lens. In order to observe the development of certain bacteria this hanging block should be examined with a microscope provided with a warm stage. With bacteria which grow readily at the temperature of the room, 20° to 22° C., an ordinary microscope will suffice.

**Examination of Fixed Material.**—For the examination of fixed material, either stained or unstained, the bacteria are spread in a thin film on the surface of a slide or coverslip and dried in the air. They are then "fixed" by the method originally devised by Koch, by passing them slowly through the flame of a Bunsen burner, usually three times. By this procedure the bacteria become adherent to the slide or coverslip. They may now be examined directly under the oil-immersion lens without staining, or stained with some of the anilin dyes. Bacteria may also be fixed in alcohol or in some of the fixatives which have been devised. Such a fixation is apt to change the form of the bacteria and make them distorted and irregular. Wet fixation may also be employed as in the Heidenhain iron hematoxylin method. In many instances the films of bacteria should be washed gently with some isotonic fluid or with tap water before examination or before staining. The débris from the culture is thus removed and better preparations are obtained. This is especially true with organisms which produce considerable amounts of viscid material in cultures or with the encapsulated bacteria. In the examination of bacteria from opaque substances

like milk, the films should be treated with a little ether or chloroform to dissolve off the fat. With films from blood it is usually advisable to dilute the material with broth or peptone solution. The corpuscles may be washed off with running water, but there is some danger that the bacteria will also be removed. Where material contains but a few micro-organisms these may sometimes be thrown down by centrifugation at a high rate of speed. By ordinary centrifugation the bacteria are not deposited because of their low specific gravity.

## STAINING REACTIONS

Bacteria are best stained by the anilin dyes which were first employed for demonstrating bacteria in tissues by Weigert and subsequently given a wide application to bacteriology by the work of Koch. These dyes are derivatives of the coal-tar product anilin, $C_6H_5NH_2$, and come in the form of powders which are monovalent acid salts of basic dyes (dye bases) or alkali salts of acid dyes. They are called basic or acid dyes as suggested by Ehrlich, although their reaction is neutral or amphoteric. By mixing acid and basic dyes neutral dyes are formed which are to be regarded as salts of the dye bases with dye acids. The free bases or acids are much less soluble and have less marked staining properties; in consequence they are not adapted to the requirements of bacteriological technic.

The important basic dyes are:

Fuchsin.
Methylene-blue.
Safranin.
Bismarck brown (Vesuvin).
Gentian-violet.
Methyl violet.
Crystal violet.

Acid anilin dyes are not suitable for the staining of bacteria. They are used in bacteriology as decolorizing reagents and as contrast stains to bring out tissue elements and differentiate them from bacteria. The principle acid dyes are:

Eosin.
Acid fuchsin.
Fluorescin.
Congo-red.

The mechanism of the staining of bacteria or other protein materials by the anilin dyes is very intricate and our present conception of the process is based almost entirely upon the ideas of Pappenheim and Michaelis. These investigators believe that each dye contains a mother substance called a "chromogen" in which lies an important complicated atom-complex called the "chromophore." The chromogens have no staining abilities in themselves and are almost neutral, having only slight acid or basic characters. The staining properties, as far as protein materials are concerned, arise from the entrance of acid or basic groups which anchor the elements to be stained and are called "auxochromes." The auxochromes are the only active ingredients which give rise to the formation of color from the chromogens. Depending on the combination of acid or basic groups with the chromogens, dyes of different acidity or alkalinity arise. For example, if basic groups combine with an acid chromophore, a weaker basic dye results than from the combination of the same basic groups with a basic chromophore.

In addition to the auxochromes, which form the absolutely essential

ingredients of the dye in staining, the dyes can take up other groups. The most important is a series of substances of acid character which form salts, nitro, sulpho, carboxyl, and nitroso groups. Of these the nitro, sulpho, and nitroso combinations always give acid character to the dyes, the carboxyl only under certain conditions. The dye molecule can also take up substances which are chemically indifferent. These increase the volume of the dye and increase the nuances of the staining reactions. Apart from the molecular volume, these reactions are dependent on the character of the chromophore, which can be colorless or stained with different degrees of intensity.

The protein material which has an affinity for and takes up the dye is called "chromophil" by Pappenheim and Michaelis. When it can be stained directly, without the application of mordants, it is usually "amphophilic," that is, has both basic (amino) and acid (carboxyl) groups in the molecule at the same time. As a result most of the stainable protein of this character takes up only one ingredient either in basic or acid dye materials. The true chemical chromophil of a protein substratum shows differential combinations when stained with neutral dye mixtures. Here an elective dissociation takes place, the basic dye combining with the protein containing acid carboxyl groups and vice versa.

Much plasma reacts as basophil. The basophil protein of the bacteria is not nuclear protein, but plastin; and, in general, basophil substance is not necessarily nuclear.

In addition to chemical staining, where there is a kind of chemical reaction between the protein materials and the various ingredients of the dyes, there is a physical staining as well; here the protein substratum is tinged as a result of diffusion and surface attraction of the staining solution. Both chemical staining and physical staining are involved in the use of anilin dyes for the demonstration of bacteria.

Bacteria differ greatly in the ease with which they are stained by the anilin dyes. Some species, like the tubercle bacillus, are very resistant. Certain constituents of the cells, like flagella and capsules, are stained with difficulty, requiring special methods for their demonstration. Spores are not stained by ordinary procedures, usually requiring the action of a mordant before the penetration of the dyestuff takes place.

**Intensity of Staining.**—The number of the side groups anchored to the dye nucleus is of the greatest importance for the intensity of the staining, that is, for the more or less firm combination of the dye with the elements to be stained, and for the resistance to decolorizing reagents. With a large number of auxophor and salt-forming groups of the same character the chemical combination with the protein materials is very firm, while with a molecule rich in indifferent groups the eventual intensity of the staining rests on the difficult diffusibility of the dye molecule provided with a greater volume. For the intensity of the staining, the chemical and physical properties of the material to be stained play a rôle as well as the purity of the dye. Chemical staining is more intense the more auxophor groups the dye possesses, and the larger the number and the greater the affinities of the corresponding chromatophil groups of the material. Physical staining is most intense when the size of the dye molecule and the pore volume of the material correspond.

**Differential staining** or color differentiation depends on the different elective affinity of the various anilin dyes for protein material and the grade of intensity of the staining. Thus the staining in solutions of dyes may be stopped when the elements with high affinity for the dyestuffs, the bacteria, are impregnated with dye and the less intensely staining tissue

elements are untouched. This is known as progressive staining. Or the less intensely stained elements like tissue cells may be decolorized by certain reagents while the more intensely stained bacteria remained stained. This is known as regressive staining. With both progressive and regressive staining several basic dyes can be used one after the other and differential staining thus brought about. This polychromatic staining rests primarily upon the elective affinity of the dyes for the protein material. It can be carried out with several stains applied one after another or with a mixture of stains. The reaction between dye and protein is both chemical and physical, and both chemical and physical processes take part in polychrome staining. The relation of the dyestuffs to each other also plays a part, some dyes acting as mordants for others and some dyes extracting others.

**Metachromatic Staining.**—Many basic anilin dyes stain the individual elements of the protein materials in the bacteria with varying degrees of intensity, *i. e.*, with different nuances of color. This is known as metachromatic staining. It is seen especially with methylene-blue and Bismarck brown.

**Decolorization** of stained material may be affected by a variety of reagents. For the decoloration to take place it is necessary that the dye be soluble in the reagent. The more soluble the dye, the easier the decolorization. The weakest differentiating reagent is water which gradually draws the dye from the preparations when it is water soluble. Alcohol is a stronger decolorizing agent, except absolute alcohol, which has no effect on dry preparations. Dilute alcoholic solutions decolorize effectively, the rapidity of action depending on the concentration. Decolorization may be brought about easily by the alternate application of water and absolute alcohol, strong diffusion currents being set up which extract the dye. Anilin oil and oil of cloves decolorize in the same way as alcohol. Glycerin is stronger than alcohol. Acids are the most powerful decolorizing reagents, the most useful being acetic acid, then hydrochloric, sulphuric, and nitric acid. The action of acids is increased by using them in alcoholic rather than in aqueous solution. The addition of hydrochloric acid to alcohol gives a more valuable decolorizing reagent than the addition of nitric acid, which oxidizes the alcohol. The decolorizing action of acids can result from a chemical decomposition of the dye or the acids can combine with a base which is soluble with difficulty and form a soluble salt with it.

The decolorizing action of water, alcohol, and acids can be exerted upon dyes which are combined chemically or physically. When a stain cannot be extracted by water or alcohol there is a genuine physical staining. When it can be extracted by acids there is a genuine chemical staining. Acid dyestuffs can be used instead of acids or an alcoholic dye be driven out by a genuine dye.

**Mordants.**—Chemical substances which bring about the staining of material with certain dyes when this material cannot be stained by ordinary methods are called mordants. True mordants are chemicals which act upon the chromatophilic elements of the protein. False mordants are substances which heighten the ability of the material to dissociate the dye salts and thus increase their diffusibility. To the false mordants belong the alkalies which cause a physical dispersion of the protein molecules. Alkalies also act chemically, decomposing the dye salts, attracting the acids, and setting free the bases for staining. Anilin oil has a similar action producing a great diffusibility of the dyestuffs, possibly by combining with them.

True mordants have a strong chemical affinity for the protein materials

and for the dyes and act by anchoring them together in a kind of indirect combination. Basic dyes require acid mordants and acid dyes basic mordants. The most characteristic action of a mordant is a change in the natural chromatophilic substance of the protein molecule, so that a dye can act which has the same character as the original material. One of the most important mordants is potassium iodid which produces a triple combination of protein materials, dye salts, and decolorizing reagents, or causes a dispersion of the dye which favors its subsequent washing out with alcohol. In Gram's stain the iodin solution containing potassium iodid forms a lasting combination with pararosanilin dyes and certain bacteria, this combination not being washed out with alcohol.

**Application.**—The anilin dyes may be dissolved in water or in alcohol and applied to bacterial films for varying periods of time. The length of time necessary for effective staining depends upon the particular dye and upon its concentration. Some dyes, like gentian-violet and fuchsin, stain bacteria rapidly, often after a few seconds' exposure. Other dyes, like methylene-blue, act somewhat slowly, requiring several minutes to impregnate the bacteria. Concentrated dyes stain more rapidly than dilute solutions. The process of staining is usually accelerated by gentle heating. In general the cell wall is stained by ordinary anilin dyes, the internal structure being demonstrated only by special methods. Permanent preparations of stained bacteria may be made by mounting the preparations in balsam. They are usually of little value since the bacteria lose their stains rapidly. Stained preparations may be freed of oil by blotting with filter-paper and kept for some time. They also have the disadvantage of fading. For the preservation of valuable material it is better to fix the films in the regular way and stain them as needed.

Pappenheim: Grundriss der Farbechemie. Berlin, 1901.
Michaelis: Einführung in die Farbstoffchemie. Berlin, 1902.
Friedberger: *In:* Handb. d. path. Mikroorg. (Kolle & Wassermann), 1. Aufl. 1903, i, 397.

## Composition and Methods of Application of the Usual Stains

### Stains for Vegetative Bacteria

Vegetative bacteria of nearly all species can be stained by aqueous and alcoholic solutions of the anilin dyes many of which have been introduced for this purpose. The composition of the stains employed varies somewhat in different laboratories and especially in different countries. The following methods are usually employed in America:

**Gentian-violet:**

*Aqueous solution:*

| | |
|---|---|
| Gentian-violet, | 1.5 grams |
| Distilled water, | 100 c.c. |

*Alcoholic solution* (saturated):

| | |
|---|---|
| Gentian-violet, | 4.8 grams |
| Alcohol (96 per cent.), | 100 c.c. |

*Carbol gentian-violet:*

| | |
|---|---|
| Gentian-violet (saturated alcoholic solution), | 1 part |
| Carbolic acid (5 per cent. solution), | 10 parts |

**Carbol Thionin-blue:**

| | |
|---|---|
| Thionin-blue, | 1 gram dissolved in 100 c.c. |
| Carbolic acid, | 1 part |
| Distilled water, | 3 parts |

Filter and stain films in the ordinary way.

**Ehrlich's Anilin Oil Gentian-violet.**—Make up two solutions, one of anilin oil in water and one of gentian-violet.

Solution No. I: *Anilin oil water:*

Add exactly 2 c.c. anilin oil to 98 c.c. distilled water in a flask; shake violently till as much of the oil is dissolved as possible. Filter twice through the same paper and keep in the dark.

Solution No. II: *Gentian-violet:*

Make a saturated solution of gentian-violet in alcohol.

Mix 75 parts of solution No. I and 25 parts of Solution No. II and filter the mixture.

Stain films in the ordinary way.

Muir and Ritchie: Manual of Bacteriology. 5. ed. New York, 1910, p. 105.

**Loeffler's Methylene-blue.**—Saturated solution of methylene-blue in alcohol, 30 c.c.

Solution of potassium hydrate in distilled water (1 to 10,000) 100 c.c.

Stain films for five minutes. Different lots of methylene-blue vary greatly in their capacity to stain bacteria, older dyes being often better than fresh dyes.

**Kühne's Methylene-blue:**

| | |
|---|---|
| Methylene-blue, | 1.5 gr. |
| Absolute alcohol, | 10 c.c. |
| Carbolic acid solution (1 to 20), | 100 c.c. |

Films stained in the regular way or decolorized with very weak hydrochloric acid made by adding a few drops of acid to a bowl of water.

**Unna's Alkaline Methylene-blue:**

| | |
|---|---|
| Methylene-blue, | 1 part |
| Potassium carbonate, | 1 part |
| Water, | 100 parts |

This solution is highly alkaline and should be diluted with 5 to 10 parts of water before using.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 74.

**Goodpasture's Polychrome Stain:**

| | |
|---|---|
| Methylene-blue (Koch f. bac.), | 1 gram |
| Potassium carbonate, | 1 gram |
| Distilled water, | 400 c.c. |

Dissolve the ingredients thoroughly and boil in a flask for five minutes. A polychrome compound of the methylene-blue is formed and most of it precipitated. After cooling add 3 c.c. glacial acetic acid. Shake thoroughly till the precipitate is dissolved and then boil gently for five minutes or until the solution is concentrated to a volume of 200 c.c. It is ready for immediate use. For frozen sections of the hardened tissue Goodpasture recommends staining with this solution for one minute or longer. The sections are then washed and mounted in water.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 54; 75.

**Neutral Red (Aqueous Solution):**

| | |
|---|---|
| Neutral red, | 1 gram |
| Distilled water, | 100 c.c. |

**Gram's Stain.**—In this stain the organisms are treated with a solution of iodin containing potassium iodid after staining with gentian-violet or methyl violet. After this treatment many species decolorize rapidly and completely in 95 per cent. alcohol, often in three to five minutes. These are known as *Gram-negative* bacteria. Others resist the decolorizing action of the alcohol, retaining the dye completely. These are known as *Gram-positive* bacteria. Owing to the fact that nearly all species of micro-organisms react regularly to Gram's stain, always retaining the stain or always decolorizing, Gram's method suffices to divide organisms into two large groups. The reaction of the bacteria to Gram's stain thus becomes of great help in identification.

*Original method:* A. For films.

1. Stain with anilin water gentian-violet solution or 2 per cent. carbol water gentian-violet solution, heating, two minutes.
2. Treat with Lugol's solution one-half to one minute.
3. Decolorize in alcohol.
4. Counterstain in some aqueous anilin dye.
5. Wash in water.

B. For sections.

1. Stain in anilin water gentian-violet solution five to thirty minutes.
2. Treat with Lugol's solution one to two minutes.
3. Decolorize in alcohol till the sections are almost colorless.
4. Wash in water.
5. Counterstain with picrocarmin or vesuvin solution.
6. Wash in 60 per cent. alcohol.
7. Dehydrate, clear, and mount.

Gram: Fortschr. d. Med., 1884, ii, 185.

**Picrocarmin Solution:**

| | |
|---|---|
| Carmin, | 1 part |
| Distilled water, | 50 parts |
| Ammonia, | 1 part |

Add picric acid till a precipitate forms. This is dissolved in a little ammonia. Now add a few drops of carbolic acid to the solution.

**Lugol's Iodin Solution:**

| | |
|---|---|
| Iodin, | 1 gram |
| Potassium iodid, | 2 grams |
| Distilled water, | 300 c.c. |

Grind the iodin and potassium iodid separately in a mortar with a small amount of water until dissolved; add balance of water.

**Weigert's Iodin Solution:**

| | |
|---|---|
| Iodin, | 1 gram |
| Potassium iodid, | 2 grams |
| Distilled water, | 100 c.c. |

Prepare by the same method as for Lugol's solution.

**Sterling's Modification of Gram's Stain.**—1. Dry films in the air and fix by heat.

2. Stain one-half minute with gentian-violet made up as follows:

Five grams of gentian-violet are ground in a mortar with 10 c.c. 95 per cent. alcohol. After solution, 2 c.c. of anilin oil are added and then 88 c.c. distilled water. The grinding is continued a short time, the mixture is allowed to stand a day or two, and filtered through paper.

3. Wash or blot.

4. Apply Lugol's solution one-half minute.
5. Wash or blot off the excess.
6. Decolorize by moving up and down in a Coplin jar containing 95 per cent. alcohol.
7. Wash in water.
8. Counterstain one-half to one minute in aqueous safranin.

Zinsser: Textbook of Bacteriology. 5. ed. 1922, p. 122.

**Paltauf's Modification of Gram's Stain.**—1. Stain three minutes in anilin water gentian-violet prepared as follows:

Add 3 to 5 c.c. anilin oil to 90 c.c. distilled water and 7 c.c. absolute alcohol. Shake thoroughly and filter through moist filter-paper till clear. Then add Gruebler's gentian-violet, 2 grams. Let stand twenty-four hours and filter from the precipitate which forms. This staining solution is good only when a metallic lustre forms on the surface and lasts four to six weeks.

2. Apply Lugol's solution two minutes.
3. Apply absolute alcohol thirty seconds.
4. Counterstain, without washing in water, in aqueous fuchsin or in weak carbolfuchsin.

Zinsser: Textbook of Bacteriology. 5. ed. 1922, p. 121. See also Sharnosky: Proc. New York Path. Soc., 1909, n. s., ix, 5.

**Nicolle's Modification of Gram's Stain.**—*For Films.*—1. Stain one to five minutes, warming, in the following solution:

| | |
|---|---|
| Saturated alcoholic gentian-violet solution, | 10 c.c. |
| Carbol water, 1 per cent., | 100 c.c. |

2. Treat with potassium iodid four to six seconds.
3. Decolorize in a mixture of 3 parts absolute alcohol and 1 part acetone.
4. Counterstain, etc.

Nicolle: Ann. de l'Inst. Pasteur, 1895, ix, 664.

**Laveran's Method for Gram-negative Bacteria.**—The films are covered with an aqueous solution of eosin made up as follows:

| | |
|---|---|
| Water-soluble eosin, | 1 gram |
| Distilled water, | 200 c.c. |

After exposure for about a minute the eosin is replaced with a saturated aqueous solution of methylene-blue which is allowed to act about thirty seconds. The films are then washed in distilled water, dried, and mounted.

Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchen's Translation, 1913, p. 210.

**Romanowsky's Method for Gram-negative Bacteria.**—1. Dry films and fix in the flame.

2. Place films in drying oven at 105° to 110° C. for about an hour.
3. Immerse films in the following staining solution, newly made up and not filtered, and leave two to ten hours:

| | |
|---|---|
| Saturated aqueous solution of Höchst's medicinal methylene-blue, | 2 parts |
| Aqueous solution of eosin, A. G. Höchst, | 5 parts |

4. Wash in distilled water.
5. Dry and mount in balsam.

Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchen's Translation, 1913, p. 210.

**Goodpasture's Stain for Gram-negative Bacteria:**

| | *gm. or c.c.* |
|---|---|
| Alcohol (50 per cent.), | 100 |
| Basic fuchsin, | 0.59 |
| Anilin, | 1.0 |
| Phenol crystals, | 1.0 |

**Albert's Stain for Diphtheria Bacilli:**

*Solution I:*

| | |
|---|---|
| Toluidin blue, | 0.15 gram |
| Methyl-green, | 0.20 gram |
| Acetic acid (glacial), | 1.00 c.c. |
| Alcohol (95 per cent.), | 2.00 c.c. |
| Water (distilled), | 100.00 c.c. |

After standing for one day the solution is filtered and is ready for use.

*Solution II:*

| | |
|---|---|
| Iodin, | 2 grams |
| Potassium iodid, | 3 grams |
| Water (distilled), | 300 c.c. |

The solution is ready for use as soon as the iodin is entirely dissolved.

**Technic of Staining.**—Smears are made on slides or cover-glasses and fixed by heat in the usual manner; stained with Solution I for one minute; washed with water; dried with good absorbent filter-paper; stained with Solution II for one minute; washed, and dried with filter-paper.

*Microscopic Picture.*—The granules of diphtheria bacilli are stained black; the bars, dark green, and the intermediate portions, a light green. Virtually all other bacteria also take a light green stain. The contrast is marked.

Albert: J. Am. M. Ass., 1921, lvi, 240.

## STAINS FOR SPORES

Owing to the hard envelope which forms on the outer layer of spores these structures are stained with difficulty. They are not attacked by ordinary dyes, which stain only the remnants of bacterial cytoplasm persisting outside of the true spore wall. For the staining of the spore itself special dyes have been devised in nearly all of which some mordant is introduced. This so affects the spore as to make possible the penetration of the spore by the stain. The time of appearance of the spores in cultures varies with the species. Some organisms like the ordinary spore-bearing species of the soil produce spores rapidly, frequently in twenty-four to forty-eight hours. With the spore-bearing anaërobes the spores appear generally after three to four days. Occasionally spores are formed slowly, after ten to fifteen days. Before spore stains are attempted the observer should make careful notes of the time at which spores appear in the various species under investigation.

**Buchner's Method.**—1. Treat the films, after fixing, with concentrated sulphuric acid thirty seconds.

2. Stain with carbolfuchsin.

Only the spores are stained.

Buchner: Aerztl. Int. Bl., 1884, xxxi, 549.

**Hauser's Method.**—1. Stain with aqueous fuchsin, passing the coverslip repeatedly through the flame forty to fifty times, continually renewing the stain.

2. Decolorize in 25 per cent. sulphuric acid.
3. Wash in water.
4. Stain with methylene-blue.

Hauser: München. med. Wchnschr., 1887, xxxiv, 492.

**Fiocca's Method.**—1. Stain three to fifteen minutes in a solution made up of 20 c.c. 10 per cent. ammonia, and 10 to 20 drops of some alcoholic anilin dye.
2. Differentiate in sulphuric acid.
3. Wash in water.
4. Counterstain with some dilute aqueous anilin dye.

Fiocca: Centralbl. f. Bakteriol. [etc.], 1893, xiv, 8.

**Klein's Method.**—This is for the purpose of allowing the material containing spores to come in contact with the dye before fixation.
1. Make an emulsion of the material containing the spores in salt solution. Add to this emulsion an equal quantity of carbolfuchsin solution.
2. Spread over coverslips, dry in the air, and fix in the flame.
3. Decolorize in 1 per cent. sulphuric acid one to two seconds.
4. Wash in water.
5. Stain in dilute methylene-blue solution three to four minutes.

Klein: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1899, xxv, 376.

**Muir and Ritchie's Method.**—Stain films with Ziehl-Neelsen's carbolfuchsin.
Decolorize in 1 per cent. sulphuric acid in water or with methylated spirit.
Wash in water.
Stain with saturated watery methylene-blue for one-half minute.
Wash in water.

Muir and Ritchie: Manual of Bacteriology. 5. ed. 1910, p. 109.

**Moeller's Method.**—1. Treat the fixed film on the coverslip with chloroform two minutes.
(Note that fat particles may be dissolved by chloroform which take the stain and later look like spores.)
2. Wash in water.
3. Treat with 5 per cent. chromic acid one and a half to two minutes.
4. Wash in water.
5. Stain with aqueous carbolfuchsin, warming the specimens, one minute.
6. Decolorize in 5 per cent. sulphuric acid five seconds.
7. Wash in water.
8. Now stain with aqueous solution of methylene-blue one-half minute.
9. Wash in water, dry, mount in balsam.

Moeller: Centralbl. f. Bakteriol. [etc.], 1891, x, 273.

**Abbott's Method.**—1. Make films in ordinary way, dry, and fix.
2. Cover with Loeffler's alkaline methylene-blue and hold over Bunsen flame till the liquid boils. Repeat this several times for about a minute.
3. Wash in water.
4. Decolorize until all visible blue color has disappeared in the following mixture:

Alcohol, 80 per cent., 18 c.c.
Nitric acid, 2 c.c.

5. Wash in water.
6. Stain three to five seconds in the following mixture:
   Saturated alcoholic solution of eosin, 10 c.c.
   Water, 90 c.c.
7. Wash in water, dry, and mount.

The spores are stained blue, the bodies of the organism rose-red.

Abbott: Principles of Bacteriology. 8. ed. 1909, p. 166.

### STAINS FOR CAPSULES

The capsules which occur regularly on certain species of bacteria, and which are occasionally produced by a large number, are usually not visible by ordinary methods of examination. When unstained they are transparent. Rarely, when well-defined capsules are produced by such species as *Bacillus coli*, they appear as minute boxes or compartments inside of which the organisms are present. Capsules are occasionally stained by such strong dyes as gentian-violet or carbolfuchsin. Frequently the capsular substance persists as a kind of unstained halo about the organisms which lie well stained within it. For the proper demonstration of capsules the capsular material must first be precipitated by some reagent like acetic acid; after this it can be penetrated by the dye. Certain media are favorable for the demonstration of capsules, particularly those containing albuminous constituents of the blood, or milk. The films in the examination for capsules should be made in blood-serum, or the medium from which the bacteria are taken should be rubbed directly on the slide or coverslip. The use of water or salt solution for the making of the films should be avoided since the capsular substance is apt to be dissolved and washed off.

**Johne's Method.**—1. Stain with warm 2 per cent. aqueous solution of methyl violet or gentian-violet one to two minutes.
2. Wash in water.
3. Decolorize in 1 to 2 per cent. acetic acid ten seconds.
4. Wash in water.
5. Examine in water, not in balsam.

Johne: Deutsche tierärztl. Wchnschr., 1894.

**Friedländer's Method.**—*For Films on Coverslips After Fixing.*—1. Treat with 1 per cent. acetic acid two minutes.
2. Wash and dry.
3. Stain with anilin water gentian-violet solution several seconds.
4. Wash, dry, mount in balsam.

*For sections:*

1. Stain twenty-four hours at 37° C. in a solution composed of:
   Concentrated alcoholic gentian-violet solution, 50 parts
   Acetic acid, 10 parts
   Distilled water, 100 parts
2. Differentiate in 1 per cent. acetic acid.
3. Wash in water and dehydrate.

Friedländer: Fortschr. d. Med., 1885, iii, 757.

**Ribbert's Method.**—*For Films on Coverslips.*—1. Stain several minutes in a solution made up of:

Dahlia (aqueous solution saturated in the heat), 100 parts
Alcohol, 50 parts
Acetic acid, 12.5 parts

2. Wash in water, dry, mount in balsam.

Ribbert: Deutsche med. Wchnschr., 1885, xi, 136.

**Vincent's Method.**—*For Coverslips from Blood.*—1. Treat one to two minutes with the following mixture to remove hemoglobin:

Glycerin, 30 parts
Cold saturated NaCl solution, 30 parts
Carbolic acid solution, 5 per cent., 6 parts

2. Wash in water.
3. Stain in a solution of carbol methylene-blue and 1 to 2 per cent. aqueous methylene-blue.
4. Wash in water and examine in water.

Vincent: Abstr., Centralbl. f. Bakteriol. [etc.], 1894, xvi, 467.

**Nicolle's Method.**—*For Sections.*—1. Stain in the following mixture:

Saturated alcoholic (95 per cent.) solution of gentian-violet, 10 parts
Carbolic acid, 1 per cent., 100 parts

2. Wash off in absolute alcohol and one-third volume acetone.
3. Wash in water, dry, embed.

**Kaufmann's Method.**—1. Stain with Loeffler's methylene-blue several hours, heating repeatedly or keeping at 37° C.
2. Wash in distilled water to which 1 to 2 drops of concentrated KOH or NaOH is added for a watch-glass of water.
3. Dry.
4. Treat with $\frac{1}{2}$ per cent. silver nitrate solution two minutes.
5. Wash again in alkaline water as above.
6. Stain with aqueous solution of alcoholic fuchsin (1 to 20) thirty seconds.
7. Wash in alkaline water.
8. Dry and mount.

Kaufmann: Hyg. Rundschau, 1898, viii, 873.

**Olt's Method.**—1. Stain with 2 per cent. safranin made in the heat and filtered, heating the films repeatedly, one-half to one minute.
2. Wash off in water and examine in water. Capsules colored yellow, bacterial bodies red-brown.

Olt: Deutsche tierärztl. Wchnschr., 1899, No. 1.

**Muir's Method.**—*Fixative* and *mordant:*

Saturated aqueous solution of corrosive sublimate, 2 parts
Tannic acid solution (20 per cent.), 2 parts
Saturated solution of potash alum., 5 parts

The films are dried in the usual manner, fixed in this solution two minutes, washed in water, then in methylated spirit, then again in water, and now stained in carbolfuchsin two to three minutes, heating gently. The films are now washed in water, placed in the mordant two to three minutes, and washed again in water. They are now stained in a saturated watery solution of methylene-blue and washed in methylated spirit.
The bacteria are stained red and the capsules faint blue.

Muir and Ritchie: Manual of Bacteriology. 5. ed. 1910, p. 110.

**Welch and Nuttall's Method.**—1. Make films in usual way on coverslips and dry in air.

2. Treat with glacial acetic acid.

3. Wash off immediately and repeatedly with saturated aqueous gentian-violet solution.

4. Wash in salt solution $\frac{1}{2}$ to 2 per cent.

5. Mount in salt solution.

Welch and Nuttall: Johns Hopkins Hosp. Bull., 1892, iii, 81.

**Huntoon's Method.**—1. Emulsify the bacteria in:

*Solution I:*

| | |
|---|---|
| Nutrose, | 3 grams |
| Distilled water, | 100 c.c. |
| Carbolic acid (2 per cent. aqueous), | 5 c.c. |

Decant into test-tubes and allow to settle.

2. Dry in the air.

3. Stain the films thirty to forty-five seconds in:

*Solution II:*

| | |
|---|---|
| Carbolic acid (2 per cent. aqueous), | 100 c.c. |
| Concentrated lactic acid, | 0.25–0.5 c.c |
| Acetic acid (1 per cent.), | 1 c.c. |
| Saturated alcoholic solution of basic fuchsin, | 1 c.c. |
| Old carbolfuchsin, | 1 c.c. |

4. Wash quickly in water.

5. Examine in oil.

As a substitute for nutrose the following milk product may be used:

Centrifugalize milk to make it fat free, add 1 per cent. NaOH, bring to a boil, cool, add ether, and shake. After a few minutes decant off the ether. The opalescent solution remaining may be used in place of the 3 per cent. nutrose.

Huntoon: J. Bact., 1917, ii, 241.

**Buerger's Method.**—1. Make films with beef, human, or other blood-serum, diluted with an equal amount of normal salt, or in ascitic or pleural fluid.

2. Treat with Müller's fluid:

| | |
|---|---|
| Bichromate of potash, | 2.5 grams |
| Sodium sulphate, | 1.0 gram |

Dissolve in 100 c.c. of water.

Saturated wtih bichlorid of mercury, 5 per cent.

3. Treat with alcohol, 80 to 95 per cent.

4. Treat with tincture of iodin U. S. P.

5. Treat with freshly prepared anilin water gentian-violet:

| | |
|---|---|
| Anilin oil, | 10 c.c. |
| Water, | 100 c.c. |

Shake, filter, and add 5 c.c. saturated alcoholic solution gentian-violet.

6. Treat with aqueous solution 2 per cent. sodium chlorid.

Buerger: J. Infect. Dis., 1907, iv, 426. Med. News, 1904, lxxxv, 1117.

**Wadsworth's Method** (*For Films and Sections Showing Pneumococci*):

1. Fix in 40 per cent. formalin two to five minutes.

2. Wash in water.
3. Stain in gentian-violet or by Gram's method.
4. Counterstain with eosin or dilute aqueous fuchsin.

Wadsworth, A.: J. Infect. Dis., 1906, iii, 610.

**Hewlett's Method.**—1. Stain the preparations by just dipping in the following solution:

| | |
|---|---|
| Carbolfuchsin, | 1 part |
| Distilled water, | 1 part |

2. Wash in water and stain for fifteen seconds in a very weak aqueous solution of gentian-violet (0.1 per cent.).
3. Wash in water, dry, and mount.

Hewlett: A Manual of Bacteriology. 7. ed. 1921, p. 127.

**McConkey's Method.**—1. Stain five minutes or more in the following solution:

| | |
|---|---|
| Methyl-green, | 1.5 gram |
| Dahlia, | 0.5 gram |
| Distilled water, | 100 c.c. |

After solution add 10 c.c. of a saturated alcoholic solution of fuchsin and make the entire volume 200 c.c. by the addition of distilled water. This stain should not be used for a fortnight and should be kept in a dark place.

2. Wash in a stream of water, dry, and mount.

McConkey: Lancet, 1898, ii, 1262.

**Hiss's Copper Sulphate Method.**—1. Make films in animal serum, preferably beef serum.
2. Dry in air and fix by heat.
3. Stain a few seconds in saturated alcoholic solution of fuchsin or gentian-violet, 5 c.c. in 95 c.c. distilled water, flooding with the dye, and holding for a few seconds over a free flame till the stain steams.
4. Wash off dye with 20 per cent. aqueous copper sulphate solution.
5. Blot (do not wash), dry, and mount.

The capsule appears as a faint blue halo about a dark purple cell body.

Hiss: J. Exper. M., 1905, vi, 317.

**Rosenow's Method.**—1. Make a thin film and when nearly dry cover with 10 per cent. tannic acid for ten to twenty seconds.
2. Wash in water and blot.
3. Stain with anilin gentian-violet by gently steaming one-half to one minute.
4. Wash in water.
5. Apply Lugol's iodin one-half to one minute.
6. Decolorize in 95 per cent. alcohol.
7. Stain with alcoholic solution of eosin.
8. Wash in water, dry, and mount.

Rosenow: J. Infect. Dis., 1911, ix, 1.

**Raebiger's Method.**—1. Dry films in air. Do not fix. 2. Stain twenty seconds in the following solution:

| | |
|---|---|
| Gentian-violet, | 10 grams |
| Commercial formol, | 100 c.c. |

Shake vigorously, allow to stand several hours, decant, and filter.

3. Wash, dry, mount in balsam.

The bacteria are stained violet, the capsules rose-violet.

Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchen's Translation, 1913, 148.

**Smith's Method.**—*For Pneumococci in Sputum.*—1. Fix fresh sputum smears by heat.

2. Steam with anilin oil gentian-violet.
3. Steam with iodin solution.
4. Decolorize in alcohol.
5. Expose a few seconds in a mixture of 4 parts of alcohol and 6 parts of ether.
6. Stain in aqueous eosin.
7. Stain in Loeffler's methylene-blue.
8. Decolorize slightly in 95 per cent. alcohol.
9. Dehydrate in absolute alcohol.
10. Clear in xylol.
11. Mount in balsam.

Wadsworth: J. Infect. Dis., 1906, iii, 610. Boston M. & S. J., 1902, cxlvii, 659.

## STAINS FOR FLAGELLA

The flagella or cilia of bacteria are stained with difficulty and many methods have been devised for this purpose. The bacterial films should be made on absolutely clean coverslips or slides, preferably after cleansing in a mixture of sulphuric acid and potassium bichromate. Great care should be exercised in making films which give a uniform distribution of the bacteria and they are best made from young cultures on plain agar, eighteen to twenty-four hours old. Even if films are made and stained with care only a small proportion give satisfactory pictures, and even in the best we are by no means certain that the arrangement of the flagella in our preparations represents accurately their arrangement in the living condition. The following methods are selected from a large number which have given satisfactory results in the hands of bacteriologists especially interested in this field of investigation.

**Loeffler's Method.**—1. Treat with the following mordant one-half to one minute, slightly warming the films.

*Mordant*

| | |
|---|---|
| Tannin solution (20 per cent. aqueous prepared in the heat), | 100 parts |
| Ferrous sulphate (cold saturated solution), | 50 parts |
| Alcoholic or aqueous fuchsin, | 10 parts |

2. Wash thoroughly in water till the mordant is completely washed out.
3. Wash in alcohol.
4. Stain with heated anilin water—fuchsin which has NaOH added to it till a suspended precipitate is formed.
5. Wash in water, dry, and mount.

*Note.*—The mordant should contain different amounts of acid and alkali, according to the species to be stained.

Loeffler: Centralbl. f. Bakteriol. [etc.], 1889, vi, 209. *Ibid.*, 1890, vii, 625.

**Bunge's Method.**—This is a modification of Loeffler's method.

1. Treat with the following mordant, one to five minutes, heating the films.

*Mordant*

| | |
|---|---|
| Concentrated aqueous solution of tannin, | 75 parts |
| Solution of liquor ferri sesquichlorati (5 per cent.), | 25 parts |
| Concentrated aqueous fuchsin, | 10 parts |

The mordant should be prepared several days before using and should be treated with hydrogen peroxid till a red-brown color is produced.

2. Wash in water.
3. Stain with heated carbol gentian-violet solution.
4. Wash in water.

Bunge: Fortschr. d. Med., 1894, xii, 653.

**Van Ermengem's Method.**—1. Treat with mordant five minutes, warming the films, or one-half hour in the cold.

*Mordant*

| | |
|---|---|
| Tannin solution (20 per cent.), | 60 c.c. |
| Osmic acid (2 per cent.), | 30 c.c. |
| Acetic acid, | 4 to 5 drops |

2. Wash in water.
3. Wash in alcohol.
4. Decolorize in $\frac{1}{2}$ to 1 per cent. silver nitrate solution one to three seconds.
5. Wash off in following solution for several seconds:

| | |
|---|---|
| Gallic acid, | 5 parts |
| Tannic acid, | 3 parts |
| Sodium acetate, | 10 parts |
| Distilled water, | 350 parts |

6. Treated with silver solution as above prepared, repeatedly moving the films about till the solution turns brown.
7. Wash in water, dry, mount.

van Ermengem: Abstr., Centralbl. f. Bakteriol. [etc.], 1894, xv, 969.

**Zettnow's Method.**—1. Apply mordant to films on coverslips by heating them on a metal plate at 80° C. in a solution made up as follows:

*Mordant*

Add to a 5 per cent. slightly warmed solution of tannin enough tartar emetic to produce a permanent precipitate. Then filter.

2. Treat with the following solution of silver, warming.

A 50 per cent. aqueous dilution of a saturated solution of silver sulphate (made from silver nitrate by addition of magnesium sulphate or sodium sulphate), to which is added 30 per cent. solution of ethylanin until the precipitate which forms is dissolved, after which more silver sulphate is added till precipitation begins.

3. Wash in water.
4. Strengthen or accentuate with a neutral solution of chlorate of gold, 1 to 2000, or with sublimate, 1 to 100.
5. Wash in water.

6. Treat with a mixture of 2 drops of a 2 per cent. soda solution and 1 to 2 drops of a solution of 1 gram pyrogallol in 20 c.c. alcohol with 2 drops of acetic acid.

7. Wash in water, dry, and mount.

Instead of treating the bacteria with silver solution they may be made visible by gold as follows. Treat the preparation after application of the mordant with neutral gold chlorate, 1 to 2000, warming till steam appears. Accentuate with a mixture of 1 drop of a 1 per cent. silver nitrate solution and 4 drops of the following solution:

| | |
|---|---|
| Water, | 150 parts |
| Citronic acid, | 2 parts |
| Pyrogallol, | 0.5 part |

By treating one minute, washing off, and again treating one minute.

Zettnow: Ztschr. f. Hyg., 1899, xxx, 95.

**Peppler's Method.**—1. Treat one to five minutes with a mordant four to six days old of the following composition:

Dissolve 20 grams of tannin in 80 c.c. hot water, after cooling add 15 c.c. of 2½ per cent. chromic acid solution.

2. Wash in water.

3. Stain with some anilin dye solution two minutes, the solution consisting of 10 c.c. concentrated alcoholic solution of the dye, 2½ c.c. carbolic acid, and 100 c.c. of water.

4. Wash in water.

5. Treat with a solution of iodin in potassium iodid one minute.

6. Wash in water.

Peppler: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1901, xxix, 345.

**Smith's Method.**—1. Treat films three minutes, warming till steam arises, in the following solution:

*Mordant*

Hot saturated solution of sublimate treated with ammonia potassium alum in excess. To 10 c.c. of this fluid add 10 c.c. of a freshly prepared 10 per cent. tannin solution and 5 c.c. carbol fuchsin. Filter.

2. Wash in water.

3. Stain three to four minutes, heating with a mixture made up of 1 c.c. alcoholic gentian-violet solution in 10 c.c. saturated ammonia potassium alum.

4. Wash in water, dry, and mount.

Smith: Brit. M. J., 1901, i, 205.

**Pitfield's Method.**—Prepare two fresh solutions as follows:

| | |
|---|---|
| A. Saturated aqueous solution of alum, | 10 c.c. |
| Saturated alcoholic solution of gentian-violet, | 1 c.c. |
| B. Tannic acid, | 1 gram |
| Distilled water, | 10 c.c. |

The solutions should be made with cold water, filtered, and preserved in separate bottles. Equal quantities are mixed before use.

1. Flood the specimens with the mixture and hold over the flame until

they nearly boil. They are then kept for one minute with the hot stain on them.

2. Wash in water.
3. Flood with anilin gentian-violet one second.
4. Wash in water, dry, and mount.

Hewlett: A Manual of Bacteriology. 7. ed. 1921, p. 131.

**Muir's Modification of Pitfield's Method.**—Two solutions are used in this method, a mordant and a stain, made as follows:

A. *Mordant:*

| | |
|---|---|
| Tannic acid (10 per cent. aqueous solution, filtered), | 10 c.c. |
| Corrosive sublimate (saturated aqueous solution), | 5 c.c. |
| Alum (saturated aqueous solution), | 5 c.c. |
| Carbolfuchsin (Ziehl-Neelsen), | 5 c.c. |

On mixing a precipitate forms which must be allowed to deposit or be centrifugalized. The clear fluid is pipetted off and preserved in a clean bottle. This mordant keeps well two to three weeks.

B. *Stain:*

| | |
|---|---|
| Alum (saturated aqueous solution), | 10 c.c. |
| Gentian-violet (saturated alcoholic solution), | 2 c.c. |

The stain should be fresh, not over two to three days old when used. The films are covered with the mordant, heated gently over the flame till steam begins to arise, allowed to steam for about a minute, and then washed well in a stream of running water for two minutes. They are now dried in the flame, covered with stain, steamed as before for about a minute, washed in water, dried, and mounted in balsam.

Muir and Ritchie: Manual of Bacteriology. 5. ed. 1910, p. 110.

**Plimmer and Paine's Method.**—1. Use cultures eighteen to twenty-four hours old at 20° C. and make a turbid suspension in water.

2. Clean slides in chromic acid and heat or roast over Bunsen burner.
3. Cool slides to blood heat, allow a drop of the bacterial suspension to flow over warm slide by which it is quickly dried, or spread with a strip of gutta-percha tissue.
4. Treat with a mordant stain made up as follows:

| | |
|---|---|
| Tannic acid, | 10 grams |
| Aluminum chlorid (hydrated), | 18 grams |
| Zinc chlorid, | 10 grams |
| Rosanilin hydrochlorid, | 1.5 grams |
| Alcohol (60 per cent.), | 40 c.c. |

Place the solids together in a mortar and before deliquescence triturate with alcohol, adding about 10 c.c. at a time. The mass goes gradually into a viscous solution of a deep red color which seems to remain permanent several years. Before using mix 1 part, about 0.5 c.c., with 4 parts of water, 2 c.c., allow to stand sixty seconds, and filter on to the slide, leaving it there for sixty seconds.

5. Wash rapidly under the tap.
6. Flood with cold carbolfuchsin five minutes, wash, dry, examine in oil.

Plimmer and Paine: J. Path. and Bacteriol., 1921, xxiv, 286.

**Letchworth Smith's Method.**—1. Make an emulsion of the bacteria and stain five to ten minutes in the following mixture:

I

| | |
|---|---|
| Tannic acid, | 1 gram |
| Potassium alum, | 1 gram |
| Distilled water, | 40 c.c. |

Dissolve by shaking or placing in an incubator over night.

II

| | |
|---|---|
| Night blue (dry), | 0.5 gram |
| Alcohol (95 per cent. or absolute), | 20.0 c.c. |

Mix the two thoroughly, remove, precipitate by filtration. The stain is good when a few days old. If not used immediately it must be filtered again before use.

2. Counterstain with aqueous gentian-violet to stain the bodies of the bacilli.

Smith: J. Med. Research, 1901–1902, vi (n. s., i.), 341.

**Shunk's Method.**—1. Transfer the bacteria from a ten- to twenty-hour culture in slant agar, or in exceptional cases from old cultures, to sterilized tap water and allow to stand fifteen to twenty minutes.

2. Place a drop of this material on a specially cleaned coverslip and without spreading allow to dry in the air.

3. Fix in one of the following solutions one-half to two minutes.

No. 1

| | |
|---|---|
| Commercial formalin (40 per cent. formaldehyd), | 1 part |
| Distilled water, | 1 part |

No. 2

| | |
|---|---|
| Chromic acid (5 per cent. aqueous solution), | 2 parts |
| Acetic acid (10 per cent. aqueous solution), | 1 part |

4. Wash in water.

5. Treat with mordants made up as follows:

Solution A

| | |
|---|---|
| Ferric chlorid (1 to 20 aqueous solution), | 1 part |
| Saturated aqueous solution of tannic acid, | 3 parts |

This solution improves with age and should be at least a week or two old. It should be filtered before using.

Solution B

| | |
|---|---|
| Anilin oil, | 1 part |
| Alcohol (95 per cent.), | 4 parts |

The films are treated with about 8 drops of Solution A, placed on the coverslip, then with 1 drop of Solution B, the combination being allowed to act two minutes.

6. Wash in water, draining off the water with filter-paper.

7. Stain two to three minutes with carbolfuchsin, 1 per cent. safranin in 50 per cent. alcohol, anilin oil gentian-violet, or Loeffler's alkaline methylene-blue.

8. Wash with water, dry, and mount in balsam.

The following stain gives especially good preparations:

| | |
|---|---|
| Saturated alcoholic methylene-blue, | 30 parts |
| Potassium hydroxid (1 to 10,000), | 100 parts |
| Solution B of mordant, | 13 parts |

This can easily be made by adding 3 c.c. of mordant solution B to 30 c.c. Loeffler's methylene-blue.

Shunk: J. Bact., 1920, v, 181.

**Adachi's Stain for the Flagella of Spirochæta Morsus Muris.**—1. Fix by exposing one-half to one minute to the vapor of osmic acid (1 gram of osmic acid and 100 c.c. distilled water with 10 drops of 5 per cent. bichlorid of mercury).

2. Stain with Giemsa's stain or with Akashi's modified Giemsa to which a little alkali is added.

Adachi: J. Exper. M., 1921, xxxiii, 647.

**Straus's Method for Staining Flagella in Living Organisms.**—1. Place a drop of broth culture on a slide.

2. Add a drop of carbolfuchsin diluted with 3 to 4 parts of water, and mix the culture with the stain.

3. Cover with a cover-glass and examine at once under an oil-immersion lens. The bacilli are stained an intense red, the flagella pale pink with deeper red points along their length. Suitable only for *Vibrio choleræ asiaticæ*, *Vibrio Finckler-Prior*, and *Vibrio Metchnikovi.*

Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchen's Translation, 1913, p. 148.

## METHODS OF DEMONSTRATING BACTERIA IN TISSUES

In the study of pathogenic bacteria, especially in the demonstration of the relationship between the micro-organisms which are obtained in cultures and the infectious processes in man and animals, it is essential to investigate the tissues carefully for the presence of bacteria. The following methods are most suitable for this purpose:

**The Weigert-Gram Method.**—The tissues should preferably be hardened in Zenker's fluid and embedded in paraffin.

1. Stain sections highly in alum-hematoxylin.
2. Wash in running water.
3. Apply 1 per cent. aqueous solution of water-soluble eosin one to five minutes.
4. Wash in water.
5. Apply anilin methyl violet one-half to one hour.
6. Wash in water.
7. Apply Lugol's solution one to two minutes.
8. Wash in water.
9. Blot with filter-paper, dehydrate, and clear in equal parts of anilin and xylol.
10. Wash off with xylol.
11. Mount in xylol colophonium.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 250.

**The Weigert-Kühne Method.**—1. Stain first in lithium carmin solution (carmin 2.5 to 5 parts to 100 parts saturated aqueous lithium carbonate) one-half hour.

2. Differentiate in alcohol or acid alcohol.
3. Wash with water.

4. Treat with crystal violet solution (concentrated solution diluted with water, to which several drops of hydrochloric acid are added, in 1 to 10 proportion) three to fifteen minutes.
5. Wash off with water or 0.6 per cent. sodium chlorid solution.
6. Dry sections on a spatula or on an object glass with filter-paper.
7. Treat with Lugol's solution one to two minutes.
8. Dry with filter-paper.
9. Decolorize with anilin oil until no more color comes out.
10. Clear in xylol.
11. Mount.

Weigert: Fortschr. d. Med., 1887, v, 228.

**Claudius' Method.**—This corresponds to Gram's method. The pararosanilin dye forms a precipitate with picric acid, which is retained firmly by certain kinds of bacteria.

1. Stain in 1 per cent. aqueous methyl-violet solution one minute.
2. Wash in water, dry with filter-paper.
3. Wash in half-saturated aqueous picric acid solutions one minute.
4. Wash in water. Dry with filter-paper.
5. Decolorize in chloroform or in oil of cloves.
6. Dry with filter-paper.
7. Embed.

Claudius: Ann. de l'Inst. Pasteur, 1897, xi, 332.

**Nicolle's Modification of Gram's Stain for Sections.**—1. Stain first with an alcoholic solution of Orth's carmin (5 parts of Orth's carmin, 1 part 95 per cent. alcohol).

2. Stain with the solution of alcoholic gentian-violet and carbol water described above in 1.

3. Treat with Lugol's solution four to six seconds.

4. Differentiate in absolute alcohol containing one-third acetone by volume.

5. Treat with 95 per cent. alcohol to which picric acid is added till a yellow-green color is formed, one to five seconds.

6. Dehydrate in absolute alcohol, clear, and mount.

**MacCallum's Combined Goodpasture and Gram's Stain.**—Fixation should be in combined formaldehyd solution and very thin paraffin sections should be used.

1. Stain for from ten minutes to one-half hour or more in Goodpasture's stain.
2. Wash in water.
3. Differentiate in 40 per cent. formaldehyd solution. This requires only a few seconds. The bright red color washes away and gives place to a clear rose.
4. Wash.
5. Counterstain in saturated aqueous picric acid. The section remains until it assumes a purplish yellow, about three to five minutes or less.
6. Wash in water.
7. Differentiate in 95 per cent. alcohol. The red reappears and some of it is washed out. Some of the yellow of the picric acid is also washed out.
8. Wash in water.
9. Stain in Sterling's gentian-violet five minutes or more.

10. Wash in water.
11. Apply Gram's iodin solution.
12. Blot, dry without washing.
13. Add anilin and xylene (equal parts) until no more color comes away.
14. Give several changes of xylene.
15. Mount in balsam.

Gram-negative organisms stain red, Gram-positive, blue. The tissue stains brilliantly in shades of red and blue.

MacCallum: J. Am. M. Ass., 1919, lxxii, 193.

## TISSUE STAINS

Occasionally it is necessary to stain fixed and embedded tissues by the methods employed in pathological histology. A few of the important stains and their method of application may be mentioned.

**Delafield's Hematoxylin:**

| | |
|---|---|
| Hematoxylin crystals, | 4 grams |
| Alcohol (95 per cent.), | 25 c.c. |
| Saturated aqueous solution of ammonia alum, | 400 c.c. |

Dissolve the hematoxylin in the alcohol, combine it with the alum solution, and expose in an open bottle to light and air for three or four days. Filter and add:

| | |
|---|---|
| Glycerin, | 100 c.c. |
| Alcohol (95 per cent.), | 100 c.c. |

Keep in the light till the color turns dark purple, filter, and keep in a tightly stoppered bottle.

Mallory and Wright: Pathological Technique, 7. ed. 1918, p. 68.

**Boehmer's Hematoxylin:**

*A*

| | |
|---|---|
| Hematoxylin crystals, | 1 gram |
| Absolute alcohol, | 10 c.c. |

Pour into a well-stoppered bottle.

*B*

| | |
|---|---|
| Potash alum, | 20 grams |
| Distilled water, | 200 c.c. |

Dissolve when warm and filter after cooling.

The two solutions are mixed after standing twenty-four hours. The resulting mixture is exposed to the air for six to seven days, kept in a well-stoppered bottle and filtered immediately before use.

Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchen's Translation, 1913, p. 218.

**Aqueous Alum Hematoxylin Solution:**

| | |
|---|---|
| Hematoxylin crystals, | 1 part |
| Saturated aqueous solution of ammonia alum, | 100 parts |
| Water, | 300 parts |

The hematoxylin crystals are dissolved in a little water by the aid of heat and then added to the other ingredients. A crystal of thymol is added to prevent the growth of molds.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 67.

**Heidenhain's Iron-hematoxylin:**

*Mordant and differentiating fluid*

| | |
|---|---|
| Iron oxyammonia sulphate, | 2.5 grams |
| Distilled water, | 100.0 c.c. |

*Stain*

| | |
|---|---|
| Hematoxylin, | 1 gram |
| Alcohol, | 10 c.c. |
| Distilled water, | 90 c.c. |

According to Park and Williams the stain should be kept in a red bottle and allowed to stand about four weeks before using. *Sections* are fixed in corrosive sublimate, Zenker's fluid or alcohol, treated with the mordant three to twelve hours, washed in water, stained in the hematoxylin twelve to thirty-six hours, washed in water and differentiated in the iron alum solution. They are finally washed in water fifteen minutes, treated with alcohol and xylol, and mounted in xylol balsam. *Films* may be fixed and stained directly.

Park and Williams: Pathogenic Microörganisms. 8. ed. 1924, p. 87.

**Mayer's Hemalum:**

| | |
|---|---|
| Hematein or its ammonia salt, | 1 gram |
| Alcohol (90 per cent.), | 50 c.c. |
| Alum, | 50 grams |
| Water, | 1000 c.c. |
| Thymol, | 1 crystal |

The hematein or its ammonia salt is dissolved in the alcohol by the aid of heat and added to the alum dissolved in the water. This solution can be diluted before use with 20 parts of water or weak alum solution.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 69.

**Alum Carmin:**

| | |
|---|---|
| Carmin, | 2 grams |
| Alum, | 5 grams |
| Water, | 100 c.c. |

Boil twenty minutes, water being added to make up for evaporation. Cool, filter, and add a crystal of thymol to prevent the growth of molds.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 71.

**Mallory's Eosin and Methylene-blue Stain.**—The tissues should be fixed in Zenker's fluid and embedded in paraffin. The sections are then stained in 5 per cent. aqueous solution of eosin for twenty minutes or longer. They may be heated in the paraffin oven for fifteen to twenty minutes to get a deep eosin stain. After washing in water they are stained in Unna's alkaline methylene-blue solution diluted 1 to 4 or 1 to 5 with water, for ten to fifteen minutes. They are then washed in water, differentiated and dehydrated in 95 per cent. alcohol until decolorization is uniform. They are finally treated with xylol and mounted in xylol balsam.

Two varieties of eosin are available: one soluble in water, and one soluble in alcohol. The saturated solutions are kept in stock with a piece of thymol added to prevent the growth of molds. Of these dilutions are made, varying from $\frac{1}{10}$ to $\frac{1}{2}$ per cent. in strength, for use after hematoxylin. When

employed before methylene-blue stronger solutions should be used, 5 per cent. or saturated.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 94.

## STAINS FOR METACHROMATIC GRANULES

Several methods have been devised for the demonstration of metachromatic granules or Babes-Ernst bodies in bacteria. Among these the most satisfactory are those of Neisser and of Piorkowski. Neisser's stain is especially suitable for the study of the diphtheria bacillus and related organisms.

**Neisser's Method.**—1. Stain three seconds or more with the following solution:

| | |
|---|---|
| Methylene-blue, | 1 gram |
| Absolute alcohol, | 20 c.c. |
| Acetic acid, | 50 c.c. |
| Water, | 1000 c.c. |

2. Wash in water.
3. Again stain with vesuvin (2 per cent. aqueous solution) three to five seconds.
4. Wash in water, dry, and mount.

Neisser: Ztschr. f. Hyg., 1897, xxiv, 443.

**Piorkowski's Method.**—1. Stain with alkaline methylene-blue, heating gently, one-half to one minute.
2. Decolorize with 3 per cent. acid alcohol (HCl).
3. Stain again with 1 per cent. aqueous eosin.
4. Wash in water, dry, and mount.

Piorkowski: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1901, xxix, 63.

## STAINS FOR CHROMATIN

Chromatin may be demonstrated in bacteria by several methods, of which the following may be recommended:

**Ziemann's Method.**—1. Stain films thirty to forty minutes in the following mixture:

| | |
|---|---|
| Methylene-blue (med. pur. Höchst, 1 per cent.), | 1 part |
| Eosin (A. G. or B. A. Höchst, 0.1 per cent.), | 6 parts |

The activity of this stain is increased by the addition of 2 to 4 parts of borax to the solution of methylene-blue. If this is done, 4 parts of the eosin solution should be employed, and the preparations should be stained eight to ten minutes.
2. Differentiate with dilute eosin or methylene-blue.
3. Wash in water, dry, and mount.

Ziemann: Centralbl. f. Bakteriol. [etc.], 1898, xxiv, 944.

**Nocht's Method.**—1. Stain five to ten minutes in the following mixture: A solution of 1 per cent. methylene-blue and $\frac{1}{2}$ per cent. soda is kept several days at 50° to 60° C. This solution is now cooled by dropping it

into a mixture of 2 to 3 drops of 1 per cent. eosin solution and 1 to 2 c.c. water until the eosin color has disappeared.

Nocht: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1898, xxiv, 839. *Ibid.*, 1. Abt., 1899, xxv, 17; 764.

**Zettnow's Method.**—1. Stain the preparations two to five minutes in the following solution:

| | |
|---|---|
| Mixture of 50 c.c. methylene-blue (med. pur. Höchst, 1 per cent.), 3 c.c. soda solution (5 per cent.), and 0.5 c.c. thymol solution (10 per cent. alcoholic), | 1 part |
| Eosin solution (B. Höchst, 10 per cent.), | 1 part |

2. Differentiate in 0.5 per cent. methylene-blue solution made slightly acid with acetic acid or in 2 per cent. eosin solution.

3. Wash in water, dry, and mount.

Zettnow: Ztschr. f. Hyg., 1889, xxx, 1.

**Michaelis' Method.**—Stain the preparations fifteen minutes in the following mixture:

| | |
|---|---|
| Methylene-blue (med. pur. Höchst, 2 grams in 200 c.c. water and 10 c.c. normal sodium hydrate), | 1 part |
| Eosin (1 to 1000 solution), | 5 parts |

Michaelis: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1901, xxix, 763.

**Reuter's Method.**—1. Fix the preparations in alcohol or in a mixture of alcohol and ether.

2. Stain fresh films twenty to thirty minutes and old films three to four hours in the following solution:

An aqueous solution of 1 per cent. methylene-blue (med. pur. Höchst) and 0.5 per cent. sodium bicarbonate is kept two to three days at a temperature of 40° to 60° C. until Nocht's red reaction appears. This is filtered in the cold, precipitated with saturated aqueous eosin to which eosin is added in excess. The precipitate is gathered on a suction filter, washed with distilled water, and dried in a desiccator or in the thermostat. A saturated alcoholic solution of this dye is made by adding about 0.2 gram to 100 c.c. alcohol, and to this 2 per cent. anilin oil is added. The final solution for staining is prepared by adding 1 to 2 drops of the stock solution to 1 c.c. distilled water.

Reuter: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1901, xxx, 248.

## STAINS FOR VOLUTIN

According to Meyer volutin can be differentiated from chromatin by its staining reactions. It stains intensely blue with methylene-blue containing 1 per cent. sulphuric acid, deep red with carbolfuchsin containing 1 per cent. sulphuric acid. Delafield's hematoxylin stains it very slowly. It decolorizes by Gram's method.

Meyer, A.: Practicum der botanischen Bakterienkunde. Jena, 1903, p. 80.

## SPECIAL STAINS FOR ACTINOMYCES

Several methods have been devised for the study of the pathogenic actinomyces (*Actinomyces bovis*, *Actinomyces asteroides*, etc.) in tissues. Among them the following may be mentioned:

**Bostroem's Method.**—1. Stain in anilin water gentian-violet solution.
2. Transfer directly to Weigert's picrocarmin solution.
3. Wash in water.
4. Treat with alcohol till the sections are red-yellow.
5. Clear and mount.

Bostroem: Ziegler's Beiträge, 1891, ix, 1.

**Israel's Method.**—1. Stain in concentrated solution of orcein in acetic acid water for several hours.
2. Wash in water.
3. Treat with absolute alcohol for several seconds.
4. Dry and mount.

Israel: Virchow's Archiv, 1886, cv, 169.

**Weigert's Method.**—1. Stain one hour in a dark red solution of orseillin in:

| | |
|---|---|
| Absolute alcohol, | 20 c.c. |
| Acetic acid, | 5 c.c. |
| Distilled water, | 40 c.c. |

2. Wash in alcohol.
3. Stain in 1 per cent. aqueous gentian-violet solution.
4. Decolorize in 60 per cent. alcohol, clear, and mount.

Weigert: Virchow's Archiv, 1881, lxxxiv, 275.

**Mallory's Method.**—1. Stain deeply in saturated aqueous eosin ten minutes.
2. Wash in water.
3. Stain in anilin gentian-violet two to five minutes.
4. Wash in normal saline solution.
5. Treat one minute with Weigert's iodin solution made up as follows:

| | |
|---|---|
| Iodin, | 1 part |
| Potassium iodid, | 2 parts |
| Water, | 100 parts |

6. Wash in water and blot.
7. Dehydrate in anilin oil.
8. Clear in several changes of xylol.
9. Mount in balsam.

Mallory: Ztschr. f. Hyg., 1895, xx, 220. Centralbl. f. Bakteriol. [etc.], 1898, xxiv, 289.

## STAINS FOR ACID-FAST BACTERIA

The bacteria belonging to the group of acid-fast organisms are not stained by the ordinary procedures. It is generally believed that the members of this group are surrounded by a kind of fatty envelope which makes the penetration of the dye difficult. The acid-fast organisms must be submitted to prolonged exposure to the dye usually with the application of heat. Certain stains like carbolfuchsin are especially valuable for this purpose. After the organisms are once stained, they resist decolorization by dilute acids or alcohol, *i. e.*, they are acid-fast or acid-resistant. Counter-stains of different color are usually used by means of which the non-acid-fast bacteria in the field are stained and differentiated from the acid-fast. A large number of stains and methods have been devised, a number of which may be employed with satisfactory results.

**Ehrlich's Method.**—1. Stain in anilin water fuchsin solution or anilin water gentian-violet solution, heating three to five minutes.
2. Decolorize in 35 per cent. nitric acid one-quarter to one minute.
3. Treat with 60 per cent. alcohol until color is no longer given off.
4. Counterstain with aqueous methylene-blue solution or Bismarck brown solution.

Ehrlich: Deutsche med. Wchnschr., 1882, viii, 269.

**Ziehl-Neelsen's Method.**—1. Stain with the following solution of carbolfuchsin, heating until steam is given off, two minutes.

*Carbolfuchsin*

| | |
|---|---|
| Crystals of carbolic acid, | 5 parts |
| Alcohol, | 10 parts |
| Fuchsin, | 1 part |
| Distilled water, | 100 parts |

2. Decolorize in 20 per cent. nitric acid five seconds.
3. Decolorize in 60 per cent. alcohol till the preparation seems colorless.
4. Counterstain in aqueous methylene-blue solution.
5. Wash in water, dry, and mount.

Ziehl: Deutsche med. Wchnschr., 1882, viii, 451.

**Fraenkel-Gabbet's Method.**—1. Stain in Ziehl-Neelsen's carbolfuchsin two minutes.
2. Decolorize and counterstain at the same time in Gabbet's methylene-blue, a saturated solution of methylene-blue in:

| | |
|---|---|
| Alcohol, | 50 parts |
| Sulphuric acid, | 25 parts |
| Distilled water, | 100 parts |

Fraenkel: Berl. klin. Wchnschr., 1884, xxi, 214.

**Czaplewsky's Method.**—1. Stain with heated carbolfuchsin.
2. Without washing in water immerse in a solution of:

| | |
|---|---|
| Fluorescin (Grübler), | 1 part |
| Alcohol, | 100 parts |

This is left for two days and poured off from the sediment, after which 5 parts of methylene-blue are added. This is now left to stand one day and poured off from the sediment.
3. Immerse ten to fifteen times in a solution of:

| | |
|---|---|
| Methylene-blue, | 5 parts |
| Alcohol, | 100 parts |

4. Wash in water, etc.

Czaplewsky: Die Untersuchung d. Auswurfes auf Tuberklebazillen. Jena, 1891.

**Dorset's Stain.**—Dorset employs a fat stain, Sudan III, to produce a specific staining of the tubercle bacillus.

Dorset: Am. Pub. Health Ass. Rep. 1898, xxiv, 157.

**Baumgarten's Differential Stain for Leprosy Bacilli.**—1. Stain in very dilute alcoholic fuchsin solution five minutes.
2. Decolorize twenty seconds in a mixture of:

Alcohol, 10 parts
Nitric acid, 1 part

3. Wash in water.
4. Counterstain with methylene-blue.

The tubercle bacillus is harder to stain than the leprosy bacillus and is not stained by this method.

Baumgarten: Ztschr. f. wissensch. Mikr., 1884, i.

**Pappenheim's Method.**—*For Differentiating Smegma Bacilli from Tubercle Bacilli.*—1. Stain a short time in carbolfuchsin heated to boiling.
2. Pour off excess of stain.
3. Without washing decolorize and counterstain by dipping three to five times in the following stain:

Corallin (rosolic acid), 1 gram
Absolute alcohol, 100 c.c.
Methylene-blue added to saturation.
Glycerin, 20 c.c.

4. Wash in water, dry, and embed.

The tubercle bacilli are stained bright red, the smegma bacilli decolorized.

Pappenheim: Berl. klin. Wchnschr., 1898, xxxv, 809.

**Herman's Stain for Tubercle Bacilli in Sputum:**

*Solution A:*
Crystal violet, 3 grams
Alcohol (95 per cent.), 100 c.c.
*Solution B:*
Ammonium carbonate, 1 gram
Distilled water, 100 c.c.

Mix 1 part of A with 3 parts of B just before using. Stain three minutes. Decolorize in 10 per cent. $HNO_3$. Wash in 95 per cent. alcohol. Counterstain in Bismarck brown.

Herman: Ann. de l'Inst. Pasteur, 1908, xxii, 92.

**Gasis' Stain.**—Add a small quantity of crystalline mercuric chlorid ($HgCl_2$) to a 5 per cent. solution of eosin (eosin 1 gram, absolute alcohol 5 c.c., water 90 c.c.), boil till the mercuric chlorid is dissolved. Cover the fixed preparations with the warm solution and heat two to three minutes over a small flame, renewing the solution as it evaporates. Wash with water, decolorize one-half minute with a 5 per cent. aqueous solution of potassium iodid, wash in 1 per cent. aqueous methylene-blue.

Gasis: Centralbl. f. Bakteriol. [etc.], 1909, l, Orig., 111.

**Kieffer's Stain.**—1. Stain by steaming one-half to three-quarters of a minute with a mixture of carbolfuchsin and 3 parts of carbol methyl violet.
2. Wash with water.
3. Treat with Weigert's iodin solution or tincture of iodin.
4. Wash with water.
5. Treat with decolorizing solution "AAA" consisting of:

Concentrated HCl, 10 c.c.
Alcohol (95 per cent.), 50 c.c.
Acetone, 40 c.c.

6. Wash with water.
7. Counterstain with methylene-blue.

**Schulte-Tigges Method.**—1. Stain one-half to two minutes with hot carbolfuchsin or twenty-four hours with cold carbolfuchsin.

2. Wash off with water.

3. Decolorize with 10 per cent. aqueous sodium sulphite solution. (This solution must be renewed every two to three days.)

4. Wash off with water.

5. Counterstain with concentrated aqueous solution of picronitric acid. The tubercle bacilli are stained red, the background light yellow.

Schulte-Tigges: Deutsche med. Wchnschr., 1920, lvi, 1225.

**Much's Methods for Staining the Tubercle Bacillus.**—Much has modified Gram's method and has demonstrated granules in the tubercle bacillus. He employs three types of staining.

*No. 1*

1. Stain in anilin water gentian-violet.
2. Treat with Lugol's solution.
3. Decolorize in absolute alcohol and oil of cloves.

*No. 2*

1. Stain in methyl violet (10 c.c. saturated alcoholic solution in 100 c.c. 2 per cent. phenol solution) by boiling over the free flame or keeping twenty-four to forty-eight hours at 37° C.
2. Treat with 5 per cent. nitric acid one minute.
3. Treat with 3 per cent. hydrochloric acid ten seconds.
4. Wash in equal parts of acetone and alcohol.

*No. 3*

1. Stain in methyl violet solution as in No. 2.
2. Treat with potassium iodid hydrogen peroxid solution (5 gr. potassium iodid in 100 c.c. 2 per cent. $H_2O_2$) for two minutes.
3. Wash in absolute alcohol.

Much: Beitr. z. Klin. d. Tuberk., 1907, viii, 85.

## STAINS FOR SPIROCHETES

Many spirochetes are easily stained by the ordinary bacterial dyes, such as gentian-violet and carbolfuchsin, or by mixtures of these dyes. In general, however, spirochetes are best demonstrated after special methods of fixation by some of the stains which have been employed with success in staining blood and certain of the protozoa. The preparations may be fixed by osmic acid vapor or by special fixatives. For staining, Giemsa's stain or its many modifications are the most valuable. In addition, special methods have been devised for such organisms as *Treponema pallidum*. Among the important fixative and staining solutions for spirochetes the following may be mentioned:

**Fixation by Osmic Acid Vapor.**—A 2 per cent. solution of osmic acid is kept in a red glass vial with a ground-glass stopper. Moist films are exposed to the fumes for a few seconds.

Park and Williams: Pathogenic Microörganisms. 8. ed. 1924, p. 88.

**Weidenreich's Method of Osmic Acid Fixation for Blood:**

| | |
|---|---|
| Osmic acid solution (osmiumtetroxyd), | 5 c.c. |
| Glacial acetic acid, | 10 drops |

This solution is put in a small glass vessel, the coverslips or slides placed on top, and exposed to the fumes for at least two minutes. The apparatus is covered with a glass bowl during this procedure. A drop of blood is now placed on the slide or coverslip and carefully spread. This is again placed on top of the dish, film side down. Fixation is complete after about one minute. If the blood films are not dry they may be dried by waving through the air. They are then passed gently through the flame three times and flooded with a solution of potassium permanganate which is left on about a minute. The solution must be very weak and light red in color. The films are then washed with water and dried with filter-paper. They may be stained with Giemsa, Ehrlich's triple stain, gentian-violet, eosin methylene-blue, or Unna's hematoxylin.

Weidenreich: Folia hæmatol., 1906, iii, 1.

**Flemming's Solution:**

| | |
|---|---|
| Osmic acid (aqueous solution 2 per cent.), | 4 parts |
| Chromic acid (aqueous solution 10 per cent.), | 15 parts |
| Glacial acetic acid, | 1 part |

*Sections* are fixed in the solution for one to three days, washed in running water five to twenty-four hours, then treated with 80 per cent. alcohol.

According to Mallory and Wright the osmic acid is best kept in a 2 per cent. solution and the chromic acid in a 1 per cent. solution. The mixture is made up fresh just before using.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 48.

**Hermann's Solution:**

| | |
|---|---|
| Osmic acid (aqueous 2 per cent. solution), | 4 parts |
| Platinic chlorid (aqueous 10 per cent. solution), | 15 parts |
| Glacial acetic acid, | 1 part |

Hermann's solution is employed in the same manner as Flemming's solution.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 48.

**Bouin's Fluid:**

| | |
|---|---|
| Saturated picric acid, | 75 c.c. |
| Formalin (40 per cent.), | 25 c.c. |
| Glacial acetic acid, | 5 c.c. |

Guyer: Animal Micrology. p. 9.

**Schaudinn's Fixative (for Protozoa):**

| | |
|---|---|
| Saturated aqueous solution of perchlorid of mercury, | 100 c.c. |
| Absolute alcohol, | 50 c.c. |
| Glacial acetic acid, | 5 drops |

The preparations before drying are fixed in the Schaudinn fluid. They are then washed for ten minutes in alcohol containing a little iodin, then in 70 per cent. alcohol for thirty to forty minutes, and stained with Heidenhein's hematoxylin.

Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchens' Translation, 1913, p. 749.

**Ehrlich's Triple Stain for Blood.**—Four different solutions are prepared as follows:

I

| | |
|---|---|
| Orange G (saturated aqueous solution), | 120–135 c.c. |
| Distilled water, | 100 c.c. |

II

| | |
|---|---|
| Acid fuchsin (saturated aqueous solution), | 65 c.c. |
| Distilled water, | 100 c.c. |
| Absolute water, | 100 c.c. |

III

| | |
|---|---|
| Methyl-green (saturated aqueous solution), | 125 c.c. |
| Distilled water, | 100 c.c. |

IV

| | |
|---|---|
| Absolute alcohol, | 100 c.c. |
| Glycerin, | 100 c.c. |

These four solutions are prepared separately and gradually mixed. The mixture must stand several weeks before using. The films are stained six to eight minutes, washed in water, dried, and mounted in balsam.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 417.

**Wright's Blood Stain:**

*Composition:*

| | |
|---|---|
| Sodium bicarbonate (0.5 per cent. aqueous solution), | 100 c.c. |
| Methylene-blue (medicinally pure), | 1 gram |

This mixture is heated in a steam sterilizer at 100° C. for one hour, put in flasks of such a size and shape that it forms a layer not more than 6 cm. deep, and then after cooling, the precipitate which forms is filtered off. To each 100 c.c. of the filtrate, which should have a deep purple when cold and viewed by yellowish transmitted light, 500 c.c. of 0.1 per cent. aqueous solution of yellowish water-soluble eosin are added. The precipitate which forms is collected on a filter, dried, dissolved in methylic alcohol (Merck's reagent) in the proportion of 0.1 gm. to 60 c.c. This staining fluid should be kept in a well-stoppered bottle to prevent evaporation and does not undergo any spontaneous change.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 418.

**Jenner's Stain:**

*A*

| | |
|---|---|
| Aqueous yellow eosin, | 6 grams |
| Distilled water (non-alkaline), | 250 c.c. |

*B*

| | |
|---|---|
| Methylene-blue (medicinally pure Höchst), | 5 grams |
| Distilled water (non-alkaline), | 250 c.c. |

Solutions A and B are combined slowly with stirring to avoid a precipitate. The material is now evaporated slowly in a porcelain dish on a water-bath at 55° C., with occasional stirring. Any paste which forms must be broken up. The resulting dried mass is ground up into an amorphous powder. The following solution is now made:

| | |
|---|---|
| Amorphous powder, | 0.5 gram |
| Methylic alcohol (Merck's reagent), | 100 c.c. |

After about three days a true solution is formed. The films are dried without fixing, stained for three minutes, washed in water, dried, and mounted.

Eyre: Bacteriological Technique. 2. ed. 1915, p. 98.

**Leishman's Stain:**

*A*

Methylene-blue (med. pure), 1 gram
Sodium carbonate (0.5 per cent. aqueous solution), 100 c.c.

This solution is kept at 65° C. for twelve hours either in a hot incubator or on a water-bath. It is then allowed to stand in a dark place at room temperature (20° C.) for ten days.

*B*

Eosin (extra B. A.), 0.1 gram
Distilled water, 100 c.c.

Solutions A and B are mixed in equal volumes and allowed to stand for twelve hours with occasional stirring. The precipitate which forms is washed thoroughly with distilled water, dried, and the following solution made:

Precipitate, 0.15 gram
Methylic alcohol (Merck's reagent), 5 c.c.

As the precipitate dissolves the alcohol is poured off, fresh alcohol added, the procedure repeated until all the sediment has been dissolved. The solution is now made up to 100 c.c. by the addition of more methylic alcohol.

The films are dried but not fixed, stained for thirty seconds. The solution is then diluted with double the volume of distilled water and mixed with a glass rod or a platinum needle. The diluted stain is allowed to act for five minutes, washed off with distilled water, some of which is left on for thirty seconds to differentiate the color. The preparation is then dried and mounted.

Eyre: Bacteriological Technique. 2. ed. 1915, p. 98.

**Giemsa's Stain:**

*Composition:*

Azur ii—eosin, 3 gm.
Azur ii, 0.8 gm.
Glycerin (Merck C. P.), 250 gm.
Methyl alcohol (Kahlbaum), 250 gm.

Films dried in air are fixed in absolute alcohol fifteen to twenty minutes. The stain is diluted by adding 1 drop to 1 c.c. distilled water containing 1 to 10 drops of a 0.1 per cent. solution of potassium carbonate. The films are covered with the diluted stain which is left on ten to fifteen minutes, then washed, dried, and mounted.

Giemsa: München. med. Wchnschr., 1910, lvii, 2976.
Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 391; 397.

**Wright's Modification of Giemsa's Method.**—In this method 10 drops of Giemsa's staining solution are shaken up gently in 10 c.c. distilled water just before use. The films are fixed in absolute alcohol or by passing gently through the flame. They are then covered with the diluted stain, warmed over the flame till a slight steam arises, allowed to cool fifteen seconds. The stain is poured off and the films again covered with fresh stain. This is repeated three or four times. The preparations are washed, dried, and mounted in balsam. The spirochetes are stained dark red.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 398.

**Burri's India Ink Method.**—Mix the material to be examined with India ink and allow films to dry. The carbon particles settle on each side of the

spirochetes (or bacteria if present) soth at the organisms appear as transparent bodies on a dark background. The best ink is known as Burri's ink or Pelikautusche No. 541. Shake well before using and dilute 1 part with 4 parts of normal salt solution or water.

Protargol 10 per cent. solution may also be used in place of the India ink.

Emery: Clinical Bacteriology and Hematology. 1917, p. 115.

**Levaditi's Stain for Spirochetes in Tissues.**—1. Fix a small piece of tissue in 10 per cent. formalin for twenty-four hours.

2. Harden in absolute alcohol twenty-four hours.

3. Wash in distilled water five minutes.

4. Immerse in a solution of silver nitrate in a ground-glass-stoppered bottle and keep in the dark for three days at a temperature of 38° C.

*Silver nitrate solution*

| | |
|---|---|
| Crystals of silver nitrate, | 1.50 grams |
| Distilled water, | 100 c.c. |

5. Wash in water.

6. Immerse twenty-four hours at room temperature in the following solution:

| | |
|---|---|
| Pyrogallol, | 4 grams |
| Formalin, | 5 c.c. |
| Distilled water, | 100 c.c. |

7. Wash in distilled water.

8. Dehydrate in 80 per cent. alcohol, then in absolute alcohol, clear in xylol, and embed in paraffin.

9. Cut, mount sections, and stain on slide for ten minutes with undiluted Giemsa's solution.

10. Wash in water and differentiate in absolute alcohol to which a few drops of clove oil have been added.

11. Wash in absolute alcohol, oil of bergamot, and xylol.

12. Mount in balsam.

Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchens' Translation, 1913, p. 731.

**Levaditi and Manouélian's Method for Staining Spirochetes in Tissues.**—1. Cut tissues into pieces 1 to 2 mm. square, fix in 10 per cent. formalin, and harden in absolute alcohol.

2. Wash in distilled water till pieces fall to bottom of dish.

3. Leave tissues at room temperature in ground-glass-stoppered bottle two to three hours, then three to five hours at 50° C. in 50 c.c. of the following solution:

| | |
|---|---|
| Aqueous solution of silver nitrate (1 per cent.), | 90 c.c. |
| Pyridin, | 10 c.c. |

4. Wash in distilled water.

5. Immerse three to four hours at room temperature in the following solution freshly prepared:

| | |
|---|---|
| Pyrogallol (4 per cent.) aqueous, | 90 c.c. |
| Pure acetone, | 10 c.c. |
| Pyridin, | 17 c.c. |

6. Dehydrate in absolute alcohol, clear in xylol, and embed in paraffin.

7. Cut stain sections on slide in 2 per cent. aqueous solution of toluidin-blue (Buschke and Fischer) and differentiate in absolute alcohol or Unna's ether-glycerin solution.

8. Dehydrate in absolute alcohol, oil of bergamot, and xylol.
9. Mount in balsam.

The spirochetes are stained black.

Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchens' Translation, 1913, p. 731.

**Benian's Congo-red Stain.**—The material containing spirochetes is thoroughly mixed on a coverslip with 1 to 2 drops of 2 per cent. aqueous solution of Congo-red. This is then spread in a thin layer, dried in the air, washed with a 1 per cent. aqueous hydrochloric acid solution, drained off at once, and dried in the air. It should not be washed in water or blotted. The spirochetes appear white and unstained in a blue background.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 399.

**Biondi-Heidenhain's Solution:**

| | |
|---|---|
| Saturated aqueous solution of orange G, | 100 parts |
| Saturated aqueous solution of acid fuchsin or rubin S, | 20 parts |
| Saturated aqueous solution of methyl-green, | 50 parts |

After making, the solutions are allowed to stand some days with an excess of dye till they become saturated. After mixing in the proper proportion the combined solution is diluted with water, 1 to 60 or 1 to 100. To make the saturated solutions about 20 grams rubin S, 8 grams orange G, and 8 grams of methyl-green should be dissolved in water.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 79.

**Fontana's Method:**

*Fixing solution:*

| | |
|---|---|
| Glacial acetic acid, | 1 c.c. |
| Formalin, | 20 c.c. |
| Distilled water, | 100 c.c. |

*Stain.*—One per cent. aqueous carbolic acid solution containing 5 per cent. tannic acid.

*Silver Solution.*—Dilute ammonium hydrate solution added drop by drop to a 0.25 per cent. solution of silver nitrate in distilled water till a faint turbidity is produced, an excess of ammonium hydrate being avoided.

The films are dried in the air without heating, washed several times with the fixing solution, washed in water, and covered with the stain. The preparations are now heated till steam arises, allowed to cool thirty seconds, washed in water, covered with the silver solution, heated again till steam arises, allowed to cool thirty seconds, dried, and mounted. The spirochetes appear brown or black.

Mallory and Wright: Pathological Technique. 7. ed. 1918, p. 399.

**Borrel's Blue.**—A few crystals of silver nitrate are dissolved in 50 c.c. distilled water in a flask of 150 c.c. capacity, which is then filled with a 10 per cent. solution of soda and shaken. A black precipitate of silver oxid forms. This is washed in distilled water several times and a saturated aqueous solution of medicinal methylene-blue (Höchst) added. The mixture is shaken several times and allowed to stand ten to fifteen days. The supernatant fluid constitutes Borrel's blue.

Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchen's Translation, 1913, p. 772.

**Laveran's Stain for Hematozoa:**

| | |
|---|---|
| Borrel's blue, | 1 c.c. |
| Eosin (water-soluble Höchst, 1 per cent. aqueous), | 5 c.c. |
| Distilled water, | 4 c.c. |

The eosin and methylene-blue solutions are filtered just before using and mixed carefully. The films are fixed ten minutes in absolute alcohol and immersed, film side downward, in a flat vessel or watch glass containing the stain. After exposure five to ten minutes, the films are washed in an excess of water, treated with a 5 per cent. aqueous solution of tannin for about two minutes, and finally washed in distilled water, dried, and mounted.

Besson: Hutchens' Translation, 1913, p. 772.

**Günther's Method.**—Dry the film by gentle heat without passing it through the flame. Cover with a 5 per cent. solution of acetic acid and leave for thirty seconds. Expose to the vapor of ammonia for a few seconds. Wash in water. Stain with Ehrlich's anilin gentian-violet for eight to ten minutes. The red blood-cells are not stained, the white blood-corpuscles and the spirochetes appear violet.

Besson: Hutchens' Translation, 1913, p. 715.

**Warthin and Starry's Method for Film-impregnation of Spirochetes.**—

1. Prepare films on No. 1 coverslips.
2. Dry thoroughly in the air.
3. Place in absolute alcohol three to five minutes.
4. Wash in distilled water. If hydrogen peroxid is used to clear background the film is placed in concentrated hydrogen peroxid for five to twenty minutes, and then washed thoroughly in distilled water.
5. Rinse cover-glass with film, in 2 per cent. silver nitrate. Cover the film side with another perfectly clean cover-glass also rinsed in the silver nitrate solution. Place the adherent pair of coverslips carefully, so as not to separate them, in a bottle of 2 per cent. silver nitrate and place in an incubator for one to two hours; then remove the cover-glasses from the silver nitrate solution and separate them.
6. Place the coverslips with the film side up in the following mixture:

| | |
|---|---|
| Silver nitrate solution (2 per cent.), | 3 c.c. |
| Warm aqueous gelatin solution (10 per cent.), | 5 c.c. |
| Warm glycerol, | 5 c.c. |
| Warm agar suspension (1.5 per cent.), | 5 c.c. |
| Aqueous hydroquinone solution (5 per cent.), | 2 c.c. |

7. After the solution is reduced remove and rinse in 5 per cent. sodium thiosulphate solution.
8. Rinse in distilled water.
9. Treat with absolute alcohol and with xylol, mount in balsam.

Warthin and Starry: J. Infect. Dis., 1922, xxx, 592.

**MacNeal's Rapid Method for Treponema Pallidum:**

| | |
|---|---|
| Saturated solution methylene-violet in methyl alcohol, | 1000 parts |
| Methylene-blue, | 1 part |
| Eosin, | 2 parts |

This stain is most easily prepared by the following formula:

| | |
|---|---|
| Methylene-violet crude (Bernthsen's), | 0.25 gram |
| Methylene-blue (med. pure), | 0.10 gram |
| Eosin (yellowish), | 0.20 gram |
| Methyl alcohol, | 100 c.c. |

The dyes are dissolved in the warm alcohol and allowed to stand at room temperature twenty-four hours before using. The films are covered with the staining solution, which is allowed to act for forty-five to sixty seconds. The preparation, with the dye on it, is quickly immersed in about 10 c.c. of a 1 to 20,000 solution of sodium carbonate, the mixture being stirred by tilting the dish. After one to two minutes the preparations are removed, washed in distilled water, dried, and mounted in distilled water. The spirochetes are intensely stained.

MacNeal: J. Am. M. Ass., 1907, xlviii, 609.

**Wright's Stain for Treponema Pallidum:**

| | |
|---|---|
| Giemsa's stain, | 1 c.c. |
| Potassium carbonate, 0.1 per cent. solution, | 1 c.c. |
| Distilled water, | 10 c.c. |

This mixture is heated to the boiling-point in a test-tube and while still hot applied to the films. After three to seven minutes the fluid becomes violet, with a thin yellow metallic scum on the surface. It is then poured off, the preparation being again covered with the hot mixture. This procedure is again repeated, the preparation then dried and mounted. The spirochetes stain intensely violet.

Mallory and Wright: Pathological Techique. 7. ed. 1918, p. 397.

## MISCELLANEOUS STAINS

**Nakanishi's Vital Staining of Bacteria.**—Nakanishi pours an aqueous methylene-blue solution (B. B. Höchst), saturated in the heat, over specially cleaned slides and washes this off till the glass has a sky-blue color, or he covers the slides with boiling hot methylene-blue solution and after drying washes it off with a towel till the slide has the right color. He then puts a drop of an emulsion of bacteria on a coverslip and lays this on the slide. All types of bacteria including the acid-fast, take up the dye, different parts of the cell taking up the dye in different intensity. Details of structure are thus apparent.

Nakanishi: Münchener med. Wchnschr., 1900, xlvii, i, 187.

**Uhma's Vital Staining of Gonococci.**—Uhma covers the slide with $\frac{1}{2}$ to 1 per cent. alcoholic solution of the dye, allows it to dry, and places on this a coverslip covered with a drop of pus.

Uhma: Arch. f. Dermaat., 1889, 1, 241.

**Plato's Vital Staining of Gonococci.**—Plato mixes a drop of pus with an oesa of a neutral red solution made up as follows:

| | |
|---|---|
| Cold saturated aqueous neutral red, | 1 c.c. |
| Physiological salt solution, | 100 c.c. |

The gonococci are stained light red and are clearly seen in the colorless or faintly stained cells.

Plato: Berl. klin. Wchnschr., 1899, xxxvi, 1085.

**Williams's Modification of Van Giesen's Method of Demonstrating Negri Bodies.**—Negri bodies may be demonstrated in *films* from the gray substance of the brain cortex near the fissure of Rolando (the crucial sulcus in the dog), from the hippocampus, and from the cerebellum. They are

partially air-dried and fixed for ten seconds in neutral methyl alcohol to which 0.1 per cent. picric acid has been added. Excess of fixative is removed by filter paper. The films are then stained in the following solution:

| | |
|---|---|
| Fuchsin (saturated alcoholic solution), | 0.5 c.c. |
| Methylene-blue (saturated alcoholic solution), | 10 c.c. |
| Distilled water, | 30 c.c. |

The smear is flooded with the stain and heated over the flame till it steams, washed in tap water, and blotted with fine filter paper. The Negri bodies are stained magenta with blue granules, the nerve cells blue, the red blood-corpuscles yellow or salmon color.

*Sections* should be fixed in Zenker's solution, embedded in paraffin, and stained by the eosin methylene-blue method.

Park and Williams: Pathogenic Microörganisms. 7. ed. 1920, p. 86.
Williams: Am. J. Pub. Hyg., 1908, xviii, 10.

**Cross's Stain for Phagocytes and Bacteria:**

*Solution A:*

| | |
|---|---|
| Distilled water (neutral), | 100 c.c. |
| Glycerin, | 20 c.c. |
| Alcohol (95 per cent.), | 20 c.c. |
| Phenol, | 2 c.c. |

To solution A are added:

| | |
|---|---|
| Crystal violet, | 0.060 gr. |
| Pyronin, | 0.200 gr. |

The material agitated to insure complete solution. This stain need not be filtered and is ready for immediate use. The films are covered with the stain, which is then washed off in distilled water. The cell nuclei are stained violet, the cytoplasm a uniform delicate lavender, the bacteria a deep purple.

Cross: Johns Hopkins Hosp. Bull., 1921, xxxii, 51.

## Chapter IV

# VITAL ACTIVITIES OF THE BACTERIA

### HABITAT

Bacteria are present everywhere in our environment, in the atmosphere, in soil, in dust, in water, and in various articles of food. They occur under normal circumstances on the skin and mucous membranes of man and animals, on the surfaces of plants. They are found in various locations as adventitious inhabitants of these areas without demonstrable significance or they survive and multiply in certain regions because of some important function which they may exercise or some property which they may possess. The soil may be regarded as the home of the bacteria whence they wander out to the atmosphere, to dust, and to water. The bacterial life there is highly diversified and the upper layers are richer in bacteria than the lower. Below 6 feet the ground becomes sterile. Various types of micro-organisms are found in the soil. The most important are the groups of nitrifying bacteria, which are of great economic value, and the nitrogen-fixing bacteria, which live on the root nodules of the legumes and enable these plants to take up nitrogen. In addition, many species of aërobic and anaërobic bacteria live normally in the soil, without serving any evident purpose. Representatives of the higher bacteria like the actinomyces are particularly abundant. Finally many different types of bacteria are constantly being deposited upon the surface of the ground from the bodies of man and animals and the soil is richer in bacteria in populated districts than in the uninhabited country. Artificially manured fields are more highly charged with bacteria than virgin soils and in them certain disease-producing organisms such as the tetanus bacillus and the "gas bacillus" of Welch and Nuttall are especially abundant.

From the soil the bacteria get into water, the number here depending upon the bacterial life in the layers through which the water has passed. Thus in surface waters like rivers and lakes the bacterial life is very abundant since these waters are constantly receiving organisms from the rich bacterial flora below. Their number is increased by rains and freshets which permit larger quantities of bacteria-laden material to be washed into water. In addition, surface waters receive bacteria from the air above, from the dust which is carried to them by currents of air, and especially from the dejecta of man and animals which may either be deposited directly or first distributed upon the soil and then given up to them later. In water coming from the upper layers the bacteria are also abundant, their number depending almost entirely upon the richness of the soil flora in the particular locality from which the water is derived. When soil contains many micro-organisms either from conditions which favor an abundant bacterial life, from the discharges of man or animals, or from artificial manuring, the quantity of bacteria which water may take up may become very large. In water taken from the deeper layers the bacteria are much less numerous because of the paucity of bacteria in these areas. Below 6 feet ground water becomes practically free from bacteria. Thus the water from true artesian wells sunk in rocks or from deep wells driven in sand is frequently sterile. In

rain water the bacteria may be very abundant, derived from the atmosphere through which the rain descends.

Bacteria are always present in the atmosphere. They are derived primarily from the soil, being lifted by currents of air from the ground as it dries and is converted into dust. Bacteria are not lifted from moist surfaces so that the number of bacteria in the air depends upon the number of viable bacteria in the dust itself. The air also receives bacteria from the exhalations of man and animals, particularly during coughing and sneezing, when minute particles of moisture containing organisms are expelled with some force from the mouth and nose. When the atmosphere is quiet and free from currents of air the bacteria are deposited in a comparatively short time upon the surfaces below, upon the soil and water in our environment, upon floors and furniture in closed spaces. When the atmosphere is disturbed by wind this deposition of bacteria takes place more slowly and the bacteria may be carried some distance before they drop to the surface beneath. The ordinary air bacteria are harmless pigmented and spore-bearing species together with yeasts and molds. The spores of pathogenic anaërobes can occasionally be found. As a rule, pathogenic organisms are present in the air only in exceptional circumstances, as in hospitals and in sick-rooms in the immediate vicinity of patients who are coughing and expectorating large quantities of infectious material, or in rooms of people with large open suppurating wounds.

In dust various types of bacteria may be present, but their number is not large. They are derived from the soil, from the discharges of man and animals, and from the atmosphere. The number of viable species is subject to great variation and depends upon the vitality of the organisms which are dried down and have become an ingredient of the dust. Many species are destroyed by the drying and cannot be recovered. Others resist drying and may be obtained regularly. The majority of pathogenic bacteria do not survive long. They may appear in the dust of sick-rooms from which the tubercle bacilli and pyogenic organisms are occasionally isolated. In rooms where many people of uncleanly habits are crowded together the dust may contain organisms from the intestinal discharges. The dust of crowded cities usually contains more bacteria than that of relatively uninhabited areas.

In consequence of this wide distribution of micro-organisms in the environment the individuals living in this environment have many bacteria deposited from time to time upon their exposed surfaces and bacteria are constantly being carried to the various cavities of the body communicating with the external world. Under normal circumstances the child before birth is entirely free from organisms and its organs and tissues are sterile. Immediately after birth bacteria are deposited upon the skin from the atmosphere, from the clothing in which the newborn child is wrapped, from the hands of attendants. With the first breath bacteria are drawn into the mouth and nose with the inspired air. At birth the entire intestinal tract is free from micro-organisms and the meconium is sterile. Almost immediately material containing bacteria of some sort or other is swallowed and in a few days various types begin to appear in the discharges. Comparatively few of the many species which are deposited upon the exposed surfaces of the body or are carried into the cavities communicating with the external world are able to survive for more than brief periods. At birth a perpetual struggle begins between the bacteria of the external world, on the one hand, and the various influences which the child may exercise against them on the other hand. As a result of this struggle only certain species are able to survive permanently on or in the body and these species con-

stitute the normal flora of the skin and mucous surfaces. Under abnormal conditions certain types of bacteria are able to multiply on the cutaneous surfaces and mucous membranes and to penetrate the protective layers which these various surfaces present. This gives rise to disease with its characteristic pathology and symptomatology.

## DISTRIBUTION

The bacteria are widely spread over the earth's surface and do not exhibit any great selective activity for certain areas or any marked limitation of distribution such as is found in plants or animals. Thus the bacteria obtained from the soil in one region are very much the same as those found in other regions. There are, however, certain restrictions due to climatic conditions. The organisms found in the water of torrid zones differ somewhat from those seen in temperate climates and in cold countries the temperature of the water is so low as to permit only a meager bacterial development. Indeed, when we consider the diseases of man and animals we find somewhat remarkable limitations. Certain diseases flourish in the tropics and die out in temperate zones, as Sir Patrick Manson has pointed out. Tropical conditions permit the development of particular types of parasites upon the surface of the body and characteristic skin infections are produced. When individuals with these diseases move to temperate or cold climates the conditions which favor the life of these parasites on the surface of the body no longer exist, and the infections heal spontaneously. In the members of Polar expeditions inflammatory conditions in the nose and throat subside after a certain zone is passed in the journey. Beyond this zone the travelers are not troubled with nasopharyngeal infections. A limitation in the distribution of bacteria by climate must be very exceptional, however, since the micro-organisms which produce disease in man and animals can flourish apparently in any area. Pyogenic infections from the ordinary pus cocci are equally common to both hot and cold climates. The viruses of tuberculosis and smallpox find conditions for development in the natives of tropical India and China and in the inhabitants of the coldest parts of Alaska and Siberia. The prevalence of certain diseases like yellow fever and East African tick fever in the tropics is to be explained, not by the inability of the parasites of these diseases to multiply in the bodies of man in particular areas, but by the lack of proper insect vectors to carry them from one individual to another and to keep the infection alive. In some instances the organisms which produce disease in man and animals survive in the environment for a brief period. Thus the soil in certain parts of Europe and South America where anthrax is prevalent in cattle and sheep may harbor the anthrax bacillus, whereas this organism does not occur to any extent in the soil of North America where the disease is somewhat uncommon. Likewise the tetanus bacillus is often in the soil of districts where tetanus is a prevailing disease of animals and man, while it is more rare in districts where this disease is infrequent. Such instances cannot be regarded as a limitation of bacterial life as such, but rather as a limitation of disease brought about by natural or artificial conditions. In general we find that the majority of the bacteria are not restricted in distribution and are able to survive and multiply in any area to which they are introduced.

## PERSISTENCE OF BACTERIAL TYPES

As far as our knowledge goes bacteria have always existed in the world much as they do at present. Our accurate information in regard to this

point does not begin before 1876 when Koch first obtained the anthrax bacillus in pure culture. Modern strains of this organism, isolated from cases of the disease in men and animals, do not differ in any essential characteristics from the strains so accurately described by Koch. We can thus say that the anthrax bacillus has not changed in fifty years. The same thing holds true for several other organisms like the typhoid bacillus and the tubercle bacillus which were first cultivated over forty years ago. The later cultures of the typhoid bacillus are identical with Gaffky's and the later cultures of the tubercle bacillus with Koch's. The higher fungi responsible for parasitic skin diseases, which were first described in 1837, were apparently the same then as the organisms isolated from these lesions today. When we go back beyond these modern times we have no definite knowledge of the types of bacteria which existed in the world. We can only say that the diseases described in early times did not differ to any extent from the same diseases as we see them today. Thus the leprosy of the Bible is not different from our present leprosy, except possibly in contagiousness, and we conclude that the same organisms were the cause of leprosy then as now. The same condition holds true for tuberculosis of man and animals. A few diseases of man, however, which are widely prevalent today apparently did not exist in ancient times and we must admit the possibility of their evolutionary spread. Whatever the origin of syphilis may be there is no satisfactory proof of its existence in Europe much before 1500 (1495) and there is uncontroverted evidence of its wide distribution over the world almost immediately after the siege of Naples by Charles VIII in 1495. We also have maladies which have died down almost to extinction at certain periods in history only to flare up later when conditions for their spread became more suitable. Thus bubonic plague, which ravaged the population of the world in the Middle Ages, almost disappeared in the 19th century, the last recorded outbreak of any extent occurring in 1843. For a period of over fifty years bubonic plague was almost unknown. Then it appeared again about 1893 in Canton, spread to Hongkong and Bombay, and then visited a large part of the civilized world. The disease had evidently remained quiescent or dormant for long periods of time, kept alive in the bodies of rodents or in man by a continuous series of non-fatal cases, and then spread again at a later period when the condition for its transmission became favorable. As far as we know no diseases have disappeared from the world and none have developed in the period of time in which medicine has been studied by modern methods. We naturally conclude that the same thesis holds true for the bacteria which produce disease.

## MUTATIONS

The determination of species in bacteria is based primarily upon morphology, the size and shape of organisms, their motility, the production of spores, and secondarily upon physiological characters, pigment production, fermentation of carbohydrates, digestion of proteins, that is, their cultural reactions. With the pathogenic organisms we test especially the capacity to produce disease or bring about well-defined lesions in experimental animals. Under artificial conditions in the laboratory bacteria differ greatly in stability. Despite constant efforts to subject bacteria to the same influences and to maintain fixed types not all species remain permanent under artificial cultivation. Changes in morphology occasionally appear and changes in physiological characters are by no means uncommon. Thus pigmented organisms like *Staphylococcus pyogenes aureus* not infrequently

lose their capacity to produce pigment after prolonged laboratory cultivation and spore-bearing organisms cease to form spores. Possibly the most marked changes observed in the laboratory relate to the phenomenon of virulence. Organisms like the pathogenic streptococci lose their virulence for animals with great rapidity, often after two or three transfers on artificial media. Rarely the characteristics by means of which we determine species are fixed upon bacteria with great firmness. Thus the original *Bacterium coli commune* of Escherich has been kept alive on artificial media for about forty years and still ferments the carbohydrates actively. Such changes as the loss of pigment production, ability to form spores and virulence are frequently permanent, and no procedures which can be applied to the bacteria are successful in bringing back the lost characters. In other cases they may be restored when the organisms are brought to greater activity, which may be accomplished by frequent plating or transfer, or when the organisms are again subjected to influences favorable for the display of the particular characteristic which has disappeared, as by frequent transfer on potato which enhances pigment production. Very rarely bacteria acquire new characteristics under laboratory cultivation. Thus the tubercle bacillus may acquire the property of producing a yellowish-red pigment and occasionally the diphtheria bacillus exhibits the same phenomenon.

Under natural conditions bacteria seem to show minor variations in properties which are like those found in artificial cultures. The typhoid bacillus isolated from the blood-stream may differ somewhat from the typhoid bacillus found in the stools of the same patient and similar variations have been described in the paratyphoid group. Such aberrant strains of microorganisms are not rare and it has always been a difficult matter to interpret them. Since our effort in the laboratory is to maintain fixed types of bacteria by subjecting our isolations to permanent and favorable conditions, comparatively little attention has been paid to degenerated laboratory strains or to aberrant forms which may be encountered in nature. The tendency of bacteria to change their properties under artificial surroundings and the frequent isolation of aberrant strains from different sources have led many observers recently to an attempt to produce artificial "variations" or "mutations" in bacteria. Many successful results have been reported and an active discussion has arisen as to whether these "variants" are to be regarded as new species or not. For practical purposes we may regard any laboratory strain of organism as a variant or mutant which has lost permanently any character which it previously possessed or which has acquired any character which it previously lacked, this change in character being carried along in subsequent generations in artificial media. Among the more important mutations for which the evidence is fairly complete the following may be mentioned:

Asporogenous strains of the anthrax bacillus can be obtained by cultivating the organism at temperatures above 42° C. or in media containing traces of disinfectants, 1 to 1000 carbolic acid, as first shown by Chamberland and Roux.[1] Similarly Migula[2] has obtained asporogenous strains of spore-forming soil bacteria by cultivating them in broth containing carbolic acid. Barber[3] has bred an asporogenous strain of *Bacillus megatherium* by selecting a single organism from a culture.

Non-pigmented strains of *Bacillus prodigiosus* were obtained by Schottelius[4] in the early days of bacteriology, usually by the selection of the colorless colonies which appear on plates and cultivation at 38° to 39° C. Similar strains were later cultivated by Goebel[5] by growing the organisms at 40.5°

to 41.5° C. These strains had completely lost their power of pigment production and this property was not restored by cultivation under influences favorable for its manifestation. Hefferan[6] has observed colorless colonies on plates from old strains of pigmented organisms and has cultivated these non-pigmented strains in succeeding generations.

Neisser[7] and Massini,[8] who have especially investigated the mechanism of mutation or variation, have found atypical strains of *Bacterium coli*. On Endo plates secondary colonies develop as yellowish-white nodules on the original white colonies. These later turn red. If the colonies be hardened *in toto* and sectioned, the nodules are seen to originate in the center of the colony and to grow through to the surface, breaking out like a malignant tumor. Massini believes that the lactose in the Endo agar, about 1 per cent., is the stimulus for the production of these secondary colonies. If the reddish nodules are transferred to fresh media a strain of organisms is obtained which throws down typical red *Bacterium coli* colonies and a few white colonies. The white colonies when transferred give rise to strains producing white colonies on the edge of which reddish secondary colonies always appear. The two strains have the same agglutination reactions. Neisser and Massini believe that this is a true mutation, a lactose-splitting organism arising from an organism which does not originally act upon this sugar. To this organism they have given the name of *Bacterium coli mutabile*.

Similar observations have subsequently been made by Bernhardt and Markoff,[9] Baerthlein,[10] and by Penfold.[11] Thus Penfold has shown that papillæ with an acid reaction appear on colonies of organisms from the intestine which do not ferment lactose actively. Subcultures from these papillæ yield lactose fermenters. Penfold regards these strains as examples of *Bacterium coli mutabile* of Neisser and Massini. Penfold has also shown that *Bacillus typhosus* produces colonies with papillæ on dulcite agar and from these acid papillæ dulcite-fermenting strains may be obtained. Rhamnose mutations of the typhoid bacillus have likewise been described by Müller.[12]

Jacobsen[13] has observed mutations in the typhoid bacillus. Organisms have been obtained from the blood in typhoid fever on Drigalski-Conradi agar which had a delayed mannite fermentation and failed to agglutinate in typhoid serum. On cultivation on lactose litmus plates small colonies appeared which on further cultivation gave rise to normal typhoid bacilli fermenting mannite rapidly and agglutinating typically. Such mutants are regarded by Jacobsen as due to the inhibition of growth produced by the Drigalski-Conradi agar. They are called *Bacterium typhi mutabile* by Jacobsen.

Variations in the capacity of bacteria to ferment carbohydrates have been artificially induced by Twort.[14] Twort has grown the typhoid bacillus, which normally does not ferment dulcite and lactose, on media containing these sugars in 2 per cent. solution. After long periods, two years or more, the organisms had acquired an ability to ferment the sugars in question. The identity of the new strains was established by agglutination. According to Penfold[15] this new capacity of the typhoid bacillus to act on lactose is rapidly lost on subsequent cultivation.

Variations in the type of colonies produced by bacteria and in the morphology of the organisms giving rise to these aberrant colonies have been seen by many observers. Thus Baerthlein has found two types of colonies on the dysentery bacillus of Shiga, pale transparent colonies made up of long slender rods often growing out into long threads, and opaque yellowish-

white colonies consisting of short, plump rods. The biochemical and serological reactions of strains obtained from these colonies are identical. After continued cultivation in the laboratory the different strains tend to throw down the same types of colonies. Similar variation in the kinds of colonies produced by bacteria have been noted by Baerthlein in *Bacillus alcaligenes*.

Jordan[16] has found a spontaneous mutation in cultures of *Bacterium coli* which were isolated by the single-cell method of Barber. Two substrains were isolated from the original strain, also by Barber's method. In one of these substrains which did not act upon saccharose at first, the ability to ferment this sugar appeared in the seventh transfer on sodium chlorid agar. This property was manifested at first by the majority of the cells, but not by all. On further transfer the non-saccharose-fermenting cells disappeared. only saccharose-fermenters being left. This newly acquired property of attacking saccharose remained permanent for over 500 generations.

Bacteria can be trained to live in concentrations of disinfectants which are destructive to the original organisms. Thus Engeland has cultivated staphylococci on agar containing 0.2 per cent. tartar emetic. In the first few days the colonies were small and delicate because of the antiseptic action of the drug. Later larger pigmented colonies appeared. Transfers from the latter grew abundantly on the antiseptic medium and finally strains developed which resisted a concentration of 4 per cent. tartar emetic. That is, strains of antimony-fast staphylococci have been artificially developed. Similar observations upon staphylococci have been made by Abbott.[17]

Among the spirochetes Gonder[18] has developed strains of *Spironema gallinarum* in the animal body which are resistant to salvarsan. This resistance was maintained for 10 to 20 passages through animals without contact with the drug and also manifested itself in the body of the insect vector, *Ornithodorus moubata*.

Mutations in cultural reactions, phagocytic, and agglutinative properties have been observed by Clough[19] in the pneumococcus. An atypical strain belonging to Type II, after six months' cultivation on blood agar, became actively phagocyted and agglutinated by antipneumococcus serum of Types I, II, and III. At the same time its cultural reactions changed, the new strain becoming bile-insoluble, failing to ferment inulin, and causing precipitation in glucose ascitic fluid agar. It also produced an intense greenish discoloration at the base of blood agar slants around the water of condensation. This strain reverted abruptly three times to its original (atypical Type II-*a*) in serological and cultural reactions. Clough further found that immune sera prepared from each variant of the strain acted strongly upon the homologous organisms, but not on the heterologous.

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## MOTILITY

In addition to the Brownian or Brunonian movement exhibited by all particles in a fluid, certain species of organisms like the cholera vibrio and the typhoid bacillus, many of the aërobic and anaërobic spore-bearing bacteria and a few micrococci and sarcinæ are endowed with the property of independent motility, moving rapidly from one place to another. This is due to the possession of special organs of locomotion known as flagella or cilia (see Chapter on Morphology). Many spirochetes are likewise motile, possibly from the presence of contractile protoplasm in their cell contents. The character of bacterial motility varies greatly with the different species as well as its rate. Thus the vibrios in general have a rapid whirling motion due to the presence of terminal cilia whose lashing movements turn the organism over rapidly and drive it from place to place. Many of the bacteria like the typhoid bacillus exhibit a progressive backward and forward motion which is brought about by the attachment of the flagella along the sides. Such organisms have no anteroposterior polarity. Finally both rotatory and progressive movements may occur in the same organism, the cilia being attached both at the ends and along the margins.

The conditions which favor or depress the motility of bacteria vary. A free supply of oxygen is generally necessary for the motility of obligate aërobes. The accumulation of deleterious products of growth tends to restrict the movements of organisms and bactericidal substances of all kinds, such as the chemical disinfectants, have a similar action. Young bacterial cells in the period of active multiplication are more motile than old organisms. During sporulation some species lose their motility, but occasionally bacteria retain it after the spores are formed within. According to Meyer and to Chester vegetative bacteria which have just emerged from spores have an especially active motility which is designated by these authors as the "swarming stage." The movements of all motile species are more marked at temperatures favorable for their multiplication.

Gabritschewsky[1] has attempted to determine the velocity of movement of different species of bacteria in cultures. Here organisms are able to travel from place to place at the rate of about 6 mm. per hour. In hanging drops the velocity is much greater. Thus the cholera vibrio moves at a rate of 0.1 to 0.2 mm. per second. This is about sixty times as rapid as in cultures.

## CHEMOTAXIS

Changes in the concentration of the chemical ingredients of substances in which bacteria are immersed have a distinct influence upon the direction of their movements. In some instances organisms are attracted to areas of higher concentration, this phenomenon being known as *positive chemotaxis*. In other cases they seem to be repelled. This is called *negative chemotaxis*. Potassium salts and peptone produce a positive chemotaxis in general while

strong acids and alkalies give rise to a negative chemotaxis. Different species of bacteria show different chemotactic reactions with different substances and chemotaxis does not seem to be related in any way to the destruction of bacteria by disinfectants or other bactericidal materials. The attraction which oxygen exerts upon certain aërobic bacteria is regarded as a positive chemotaxis and its antagonistic action upon anaërobes as a negative chemotaxis.

## RATE OF MULTIPLICATION

It has already been pointed out that bacteria multiply primarily by binary fission and that there is still some discussion as to whether spore-formation serves for an actual increase in the number of organisms or not. It has been suggested that granules of an indefinite character are given off by bacteria and develop into mature vegetative forms, but the evidence in favor of this view is not convincing. The process of binary fission can be watched under the microscope and the segmentation of one organism into two daughter individuals can be exactly timed. Buchner, Longard, and Riedlin[2] estimate the length of a single generation, that is, the time which elapses between the origin of one cell and its segmentation into two fully formed descendants, by the following method:

a = number of bacteria originally present
b = number of bacteria after a definite time (T)
n = number of generations

If multiplication takes place by binary fission then the law of geometrical proportion holds, represented by this equation:

$$b = a \times 2n$$

If this be put in logarithms we have the following:

$$n = \frac{\log^b - \log^a}{\log^2}$$

The length of a generation G equals $\frac{T}{n}$.

Based upon this formula a generation of the cholera vibrio in sugar broth at 37° C. has been found to last from nineteen to forty minutes, that of the typhoid bacillus in broth at 37° C. from twenty-nine to thirty-three minutes. According to Barber the generation time of *Bacillus subtilis* at 37° C. is about twenty minutes.

When organisms are transferred from old to fresh media a definite period usually elapses before multiplication begins. This is about two hours for the typhoid bacillus, for example. This time before multiplication becomes active is known as the lag period or bacterial lag. When transfers are made from very young two- to three-hour cultures multiplication may begin immediately, no lag period being noticeable. When older material is employed the lag period is appreciably lengthened. Chesney,[3] who has investigated the phenomena of bacterial growth in great detail, differentiates four different phases in the multiplication of bacteria when transferred to new media, the latent period or lag, the phase of maximal growth, the stationary period and the phase of decline. The lag or latent period does not appear when transfers are made from bacteria growing at their most rapid rate, but rather when transfers are made from cultures in the stationary period or phase of decline. Chesney believes that the lag or latent period is due to the death of some of the organisms which are carried over to the new media, probably because of an injury which the bacterial cells have suffered in their previous environment.

Sherman and Albus[4] have shown that young bacterial cells are more sensitive to harmful agencies than mature forms. These authors suggest that in the lag period the old cells must undergo a kind of biologic rejuvenescence which fits them for reproduction before the culture can enter the phase of rapid growth.

The influence of temperature upon the multiplication of bacteria is very marked. Thus Barber[5] has isolated strains of *Bacterium coli* by the single-cell method and studied their multiplication at different temperatures, using the Buchner formula for calculating his results. Reproduction of this organism begins at about 10° C., increases markedly up to 37° C. at which temperature the maximum rate is nearly seventeen minutes. This rate of reproduction remains constant between 37° C. and 45° C., then it falls rapidly, practically ceasing at 49° C.

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## RANGE OF REACTION

Bacteria multiply within wide limits of acidity and alkalinity and these limits mark the range of reaction in which bacteria flourish. In general they grow best within a narrow range of reaction and the hydrogen-ion concentration which marks the limit of active multiplication has now been determined for our most important species. In culture media in which organisms are multiplying the reaction varies greatly at different periods from the changes brought about in the ingredients of the culture material by the growth of organisms, chiefly by the action of bacterial enzymes. Thus many organisms produce considerable quantities of acids from carbohydrates, the acidity becoming so great that growth is inhibited and the organisms even killed. Other species may produce similar quantities of acids from these carbohydrates, but are able to resist the destructive action of the acids. They then attack the protein materials in the media and produce alkaline substances which overcome the acidity and bring the reaction back to neutral or render it alkaline. Bacteria are inhibited or killed more quickly by acidity than by alkalinity and many species are destroyed by small quantities of acid, especially by mineral acids, while they may resist the destructive action of organic acids.

## PHYSICAL CONDITIONS AFFECTING BACTERIA

**Temperature.**—Bacteria develop and flourish best at various temperatures and exhibit a marked capacity to multiply and to survive at different degrees of heat. The temperature at which any organisms multiply most vigorously is called the *optimum temperature* for that species. Below and above this the multiplication is less marked. For the majority of species which interest the student of medicine and hygiene the optimum temperature is about 37° C., the heat of the body. Water bacteria and many of the organisms found in soil and dust grow better at a somewhat lower temperature, about 22° C. or that of the ordinary room. Between 37° and 22° C. practically all the important species develop satisfactorily. As the temperature is increased above 37° C. many organisms fail to multiply. Thus the gonococcus will not grow above 39° or 40° C. The greatest degree

of heat at which organisms are able to multiply is known as the *maximum temperature* for that species. Many organisms which have an optimum temperature of about 37° C. are able to grow at temperatures considerably above this. Thus *Bacterium coli* develops abundantly at 42° to 43° C. and somewhat even at 47° to 48° C. The spore-bearing bacteria of the soil which grow vigorously at the temperature of the body frequently develop at higher temperatures, often up to 50° C. The anthrax bacillus grows well at 44° C., but loses its capacity to sporulate. *Bacillus subtilis* grows well at 48° C. Above 50° C. the ordinary bacteria fail to multiply. Certain species, however, thrive at 50° to 70° C. at which temperatures ordinary protein is coagulated. These species are known as *thermophils*. They occur in the waters of thermal springs and in the soil where they usually exist as spores, vegetating only under such special conditions as are furnished by decaying manure pits and fermenting silo pits. Some of the thermophils are obligate, requiring a high temperature for their multiplication, while others are facultative, developing both at high temperatures and at lower temperatures, about that of the body.

As the amount of heat to which bacteria are exposed is increased a point is reached at which the organisms are killed. This is known as the *thermal death-point.* It varies with different species and with the stage in the life history. Thus vegetative rods are destroyed at much lower temperatures than spores, many of which are extremely resistant and are killed only at the boiling point of water or somewhat above this. The time of exposure to the heat is also a factor and occasionally long continued action is necessary for the destruction of both vegetative rods and spores. The majority of vegetating cells are killed at temperatures ranging from 55° to 65° C., with an exposure of twenty to thirty minutes.

As the temperature is lowered the multiplication of bacteria becomes less active. The least heat at which they can grow is known as the *minimum temperature* for that species. Below 20° C. the number of strains which can multiply becomes less and less and the majority cease to vegetate or vegetate very slowly at 16° C. As the temperature drops below this point bacteria go into a kind of dormant state in which they do not multiply but survive long periods. Thus many pathogenic organisms, like the pneumococcus and streptococcus, remain alive for months and even years at the temperature of the ice-box, about 6° C., provided they are protected from drying. The typhoid bacillus can apparently survive for weeks in ice. In food products kept at low temperatures the number of surviving bacteria is often surprisingly large. Frequently ice-cream contains many viable bacteria. Even extremely low temperatures, such as are produced by liquid air, do not always kill micro-organisms.

**Light.**—Bacteria flourish better in the dark than in the light and direct sunlight destroys nearly all types. This destructive action is difficult to separate from desiccation and the action of sunlight and desiccation is usually a combined one. Certain species are especially susceptible to the influence of sunlight and drying. Thus the cholera vibrio is destroyed in four hours by exposure to the sun's rays in tropical India. The ultraviolet rays of light are inherently bactericidal and bacteria in a moist medium are destroyed by them with some rapidity. Thus the bacteria in water are easily killed by exposure to the action of the ultraviolet light (see Chapter VI).

**Electricity.**—The electric current has little influence upon bacteria. If strong currents of electricity are passed through solutions containing micro-organisms, many of them are destroyed. This is usually ascribed

to the heat which is generated, although electrolytes may be produced which have a bactericidal action.

**Osmosis.**—It has already been pointed out that the bacterial cell consists of a mass of protoplasm or cytoplasm enclosed in a thin delicate membrane. Chemically the cell is an aggregate of many simple or highly complex materials, partly colloid and partly crystalloid. These dissolved substances exert a distinct osmotic pressure from within upon the cell-wall and under normal circumstances this pressure is equal to the osmotic pressure from without by the materials which surround the bacteria. Slow changes in the external pressure are met by a compensatory change in the pressure within the cell. If the variations in the outer pressure are sudden, however, morphological changes occur in the bacteria and these are designated as *plasmolysis* and *plasmoptysis.*

Plasmolysis occurs when the bacteria are immersed suddenly in solutions of substances which tend to draw water away from the cell contents, such as higher concentrations of salts, glycerin, lecithin, etc. The cell-wall acts as a semipermeable membrane and salts pass into the interior of the cell as the water escapes. With this loss of water the cytoplasm shrinks away from the cell wall and aggregates of highly refractile granules are formed. Fischer has studied this phenomenon in great detail and finds that different species of bacteria are plasmolyzed by different salt concentrations. Thus the anthrax bacillus is not plasmolyzed by 2 per cent. NaCl solution while cholera vibrios, typhoid bacilli and *Bacillus pyocyaneus* are rapidly affected. Older bacteria are plasmolyzed more easily than young cells. The polar staining of organisms like the plague bacillus and the members of the hemorrhagic septicemia group is regarded by Gotschlich[2] as the result of plasmolysis occurring spontaneously under artificial conditions. In plasmolysis the cells are injured, but not necessarily killed. If the external osmotic pressure be reduced again, as by immersion in a solution of salt which is normal to the cells, water gradually accumulates in the interior and the contracted protoplasm again swells to its usual state.

Plasmoptysis occurs in bacteria when the external osmotic pressure is lowered suddenly as by immersion in distilled water. The rapid passage of water through the cell membrane as a result of the higher concentration of salts within the cell produces an increased internal osmotic pressure. The bacteria first swell up and then burst. The cytoplasm is converted into a mass of small glistening aggregates which are released from the cell and eventually pass into the surrounding medium. In plasmoptysis the original bacteria are necessarily killed. It has been suggested that the masses of freed cytoplasm are viable and may regenerate a new cell wall. Evidence in favor of this revolutionary view is lacking.

1. Fischer, E.: Vorlesungen über Bakterien. 2. Aufl., 1903.
2. Gotschlich: *In:* Handb. d. pathog. Mikroorg. (Kolle and Wassermann). 2. Aufl., 1912, i, 30–292.

## FILTRATION

Bacteria pass rapidly through the pores of ordinary filter paper, the rapidity of passage depending chiefly on the size of the pores. With finely-meshed paper the larger organisms are held back, especially if there be a certain amount of albuminous material in the solution to collect on the filter and help to clog it. For the purpose of completely removing bacteria from solutions special filters are used, the Pasteur-Chamberland bougies or candles of unglazed porcelain or the Berkefeld filters made of diatomaceous earth.

These are made in various sizes and must be used with positive pressure applied to the solution containing the organism or this solution must be drawn through the filter by suction. Great care must be exercised in this type of filtration since the candles are apt to crack and permit bacteria to escape through very minute openings. With certain types of bacterial suspensions, especially if the micro-organisms are very abundant, the filters clog rapidly and a mass of viscid slimy material collects on the outer surfaces.

The mechanism by means of which bacteria are removed from solutions by Chamberland or Berkefeld filters has been carefully investigated and several factors are involved. If a cross section of the filter be made and examined microscopically the thin mass is found to contain many irregularly limited hollow spaces which vary greatly in size and may be 2 to 100 $\mu$ in diameter. In addition numerous fine canals or pores are found in the filter substance communicating with the hollow spaces. Retention of particulate matter takes place in these canals or pores. If a filter which has been used for removing Gram-positive bacteria from a solution be sectioned and stained by Gram's method, the organisms are found not in aggregates but distributed in various areas of the compact substance. One can thus get an approximate idea of the fineness and disposition of the capillary anastomoses. Rosenthal[1] believes that the positive or negative pressure applied in filtration drives the fluid with its suspended particles against the pores and channels and makes them go through the filter mass by the shortest route. There is always some place which offers resistance and is stopped up by the organisms or particulate matter. Rosenthal regards the width of such a space as the "determining pore diameter" (wirksame Porengrösse) and believes that the passage of organisms through the filter depends on this diameter and the dimensions of the bacteria. His observations indicate that the pore size of bacteria-tight filters is from 0.5 to 2 $\mu$. Schmidt[2] estimates it for Berkefeld filters at 0.2 to 0.8 $\mu$.

In addition to the influence exerted by the diameter of the canals or pores which traverse the filter, the filter mass itself apparently plays some rôle, possibly through molecular attraction and adsorption. Finally certain characteristics of the material to be filtered are of moment such as the form and elasticity of the elements to be removed, the viscosity of their surfaces, the character of the medium surrounding the elements and its content in protein or dissolved colloid material.

Neither Berkefeld nor Chamberland filters offer an *absolute impermeability* to the passage of micro-organisms. Minute bacteria like *Spirillum parvum* of Esmarck and *Micrococcus Mesnili* of Borrel pass readily through bougies which were formerly regarded as able to hold back all particulate matter. Rosenthal thinks that these organisms grow around the impervious stenoses in the filter substance. Smith and Wherry[3] have found that the bacillus of guinea-pig pneumonia passes through Berkefeld filters. The motility of micro-organisms is an important factor in addition to their size. The small actively motile anaërobic bacteria in the mouth are extremely difficult to remove from solutions by any type of filtration. The passage through the filter substance may also be determined to some extent by the character of the motility. Thus certain spirochetes pass readily through Berkefeld filters. Wolbach and Binger[4] have shown that *Leptospira biflexa* passes a Berkefeld V bougie and *Spirochæta elusa* through Berkefeld V, N, and W. Noguchi[5] has reported that *Leptospira icteroides* can be filtered through a Berkefeld V or N. Possibly the wriggling boring motion of these spirochetes enables them to traverse tortuous spaces which

would be clogged up rapidly by ordinary bacteria. Schmidt states that the width of organisms and not their length is the important factor which determines their ability to pass filters, the maximum width for filtration being about 0.2 $\mu$.

1. Rosenthal, W.: Centralbl. f. Bakteriol. [etc.], 1908, 1. Abt., xlv, Orig., 563–573.
2. Schmidt, P.: Ztschr. f. Hyg., 1910, lxv, 423–434.
3. Wherry, W. B.: J. Med. Research, 1902, viii (n. s. iii), 322–328.
4. Wolbach, S. B., and Binger, C. A. L.: J. Med. Research, 1914, xxx (n. s. xxv), 9; 23.
5. Noguchi, H.: J. Exper. Med., 1919, xxix, 547–564.

## FOOD REQUIREMENTS OF THE BACTERIA

The chemical analysis of the bacteria has indicated that they are essentially water plants the protoplasm of which contains the chemical elements found in other types of plant protein. In order to build up their cells in the anabolic phase of their activity the bacteria must find the ingredients of their protoplasm in their immediate environment. The most important elements which they require for assimilation are hydrogen, oxygen, nitrogen, carbon, sulphur, phosphorus, potassium, calcium, magnesium, and iron. The *hydrogen* is obtained from the water in the various substances upon which the bacteria live. They do not multiply on dry surfaces, but the amount of moisture necessary for their development may be very small. Thus certain pigmented species like *Bacillus prodigiosus* grow on dry bread which contains only minute traces, but this amount suffices for a sparse multiplication. When more moisture is available bacterial life may become luxuriant. Micro-organisms develop abundantly in fresh water and in sea-water, in milk, in moist foodstuffs and in tissue fluids. The *oxygen* is derived from the atmospheric oxygen which occurs in loose combination in the diverse substances which serve to maintain the life of the organisms or it is taken from some of the complex organic compounds in which they multiply. The *nitrogen* comes from the atmospheric nitrogen or from nitrogenous compounds in the environment. The *carbon* is derived from the organic materials, particularly the proteins and the carbohydrates, in the menstruum surrounding the bacteria. Finally the mineral elements are always present in this menstruum and may be assimilated by bacteria in a number of ways. In the changes induced by bacteria in the various substances surrounding them energy is liberated and utilized by the bacteria in building up their cytoplasm.

The simple principles just indicated do not, however, explain the manifold activities which the bacteria exhibit during their anabolic life. Many different types of bacteria have been described which have special properties by means of which they utilize the chemical substances in their environment and build them up into their cytoplasm. This is clarified if we consider the relation of these bacteria to various substances in detail.

**Oxygen.**—All bacteria apparently require oxygen and give up carbon dioxid during their life activity. That is, they have a true respiratory exchange like other types of protoplasm. This has been demonstrated for the aërobic species by Lubbert[1] and by Hesse.[2] Frederick[3] has recently shown that a species of aspergillus, closely similar to the bacteria in its vital processes, also takes up oxygen and gives off carbon dioxid. More oxygen is absorbed than is represented by the carbon dioxid evolved and the excess of oxygen is apparently utilized by the bacteria in building up their cytoplasm. Anaërobic bacteria also give off carbon dioxid, possibly from a true metabolic activity of the cells (intramolecular respiration). Many species

are able to utilize the atmospheric oxygen in the medium in which they live and are not inhibited by its presence. These species are called *aërobic bacteria.* Other types are unable to live in the presence of atmospheric oxygen and obtain this element by breaking down the diverse compounds in their environment. These are called *anaërobic bacteria.* Certain organisms are very exacting in their relation to oxygen and are inhibited by small quantities. These are spoken of as *obligate anaërobes.* They multiply at times in materials containing oxygen, but in such instances other species of micro-organisms are usually present to utilize the oxygen and create true anaërobic conditions.

Finally many species multiply equally well in the presence or absence of atmospheric oxygen, being able to utilize the oxygen present in the menstruum surrounding them and to take oxygen from this menstruum by breaking down some of its complex compounds. These species are called *facultative anaërobes.* Other organisms actually require the presence of atmospheric oxygen and are inhibited by its absence. These species are sometimes called *obligate aërobes.*

The relationship of bacteria to oxygen is very complicated and there are still many unexplained observations. Thus there is a wide range in the amount of oxygen which not only permits the development of bacteria, but favors it. This is usually referred to as *oxygen tension.* Certain anaërobes which originally multiply only in the absence of atmospheric oxygen can be so accustomed to the presence of small quantities, that is, to a low oxygen tension, that a continued development becomes possible. With other species like the typhoid bacillus and the gonococcus, which are usually able to multiply in the presence of atmospheric oxygen, its partial exclusion leads to a more luxuriant growth.

**Carbon Dioxid.**—Bacteria produce carbon dioxid as a result of true respiratory exchange. The amount of carbon dioxid so produced is very small. Large quantities of this gas are frequently evolved by bacteria from the breaking down of complex materials in their environment which may serve as food and from which change energy may be liberated. Thus a number of bacterial types decompose solutions of carbohydrates and liberate carbon dioxid just as is accomplished by the yeast cells. Other species break down protein material and carbon dioxid is evolved in this process.

**Carbon Monoxid.**—Certain rare species of bacteria can live in an atmosphere of pure carbon monoxid. These have been described by Beijerinck and van Delden[4] and Jensen[5] and are known as "carboxydomonas." They secure their growth energy by the oxidation of carbon monoxid, forming carbon dioxid.

**Nitrogen.**—This gas has usually no effect upon the development of bacteria and many species of facultative aërobes and anaërobes are able to multiply in almost a pure atmosphere of nitrogen. Certain species known as "azotobacter," which depend primarily for growth energy upon the oxidation of carbohydrates, are capable of fixing atmospheric nitrogen when grown in solutions containing carbohydrates and deficient in combined nitrogen. Other species now known as "rhizobium" (*Bacillus radicicola*) are capable of fixing atmospheric nitrogen when grown in the presence of carbohydrates and in the absence of compounds of nitrogen. Such species are found in the root nodules of leguminous plants.

**Hydrogen.**—Many species of bacteria evolve hydrogen from the decomposition of organic matter or from the fermentation of carbohydrates. Three species have been described, one by Kaserer[6] and two by Niklewski[7] which grow in the absence of organic matter and secure growth energy by

the oxidation of hydrogen with the formation of water. These are now called "hydrogenomonas."

**Methane.**—Certain rare organisms have been described by Söhngen[8] and by Orla Jensen which grow in the absence of organic matter and oxidize methane, producing carbon dioxid and water. They are known as "methanomonas."

**Alcohol.**—Many organisms grow in alcoholic solutions as obligate aërobes, oxidizing the alcohol to acetic acid. These species are now designated "acetobacter."

**Ammonia.**—Certain organisms known as "nitrosomonas" oxidize ammonia to nitrites and thereby secure growth energy.

**Nitrites.**—Certain species oxidize nitrites to nitrates. They are called "nitrobacter."

**Carbon** is obtained by bacteria under special circumstances from the carbon dioxid of the atmosphere and possibly from carbon monoxid. As a rule this element is derived from the complex carbohydrates and proteins in their immediate environment. The sugars, starches, alcohols, peptones, and amino-acids are all attacked by bacteria and carbon compounds are liberated and apparently utilized by the organisms. The chemical changes in this breaking down of carbohydrates and proteins may liberate considerable amounts of energy and undoubtedly all these transformations contribute to the building up of the bacterial protoplasm during the anabolic phase. The ability of bacteria to attack these substances extends far beyond their needs, and profound changes are brought about by various enzymes. This is usually regarded as an evidence of catabolic activity on the part of the bacteria. There is no very sharp line of demarcation and it is frequently impossible to say whether the changes induced by bacteria in carbohydrates and proteins serve primarily for the purpose of securing growth energy or are merely the result of the action of excretory bacterial products. In many cases substances may be produced which are distinctly antagonistic to the bacteria, sometimes inhibiting their growth, and sometimes even killing them.

## FERMENTATION

The term "fermentation" was originally applied to any type of bacterial activity in which complex carbohydrates or nitrogenous substances were broken down to simpler compounds. It was almost synonymous with the term "decomposition." The term "putrefaction" was employed for the breaking down of nitrogenous materials with the evolution of foul-smelling products, the term "decay" to a similar decomposition in which such foul-smelling products were not noticeable. Chiefly as a result of the work of Fischer,[9] the term "fermentation" has come to be applied to that type of bacterial activity in which carbohydrates are attacked, the term "putrefaction" to a similar attack upon protein materials. In this sense putrefaction is practically identical with proteolysis. Certain bacteria split up nitrogenous materials with the liberation of foul, stinking gases characteristic of cadaveric decomposition and this change is often called putrefaction. It is simpler to regard all changes produced by bacterial activity in proteins as putrefaction, since the actual production of foul odors by bacteria is only a part of the entire process of protein decomposition. The principal types of fermentation are the following:

**Alcoholic Fermentation of Sugars.**—The fermentation of sugar with the production of alcohol has been studied especially with the yeasts, the most important of which is *Saccharomyces cerevisiæ*, certain strains being able to

produce large quantities of alcohol. This property is shared by many molds and by a few bacteria. With the yeasts the reaction is usually a splitting of the hexose sugar into ethyl alcohol and carbon dioxid according to the following formula (Buchanan[10]):

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} = \underset{\text{Ethyl alcohol.}}{2C_2H_5OH} + \underset{\text{Carbon dioxid.}}{2CO_2}$$

In his original study of the fermentation of sugar to ethyl alcohol and carbon dioxid Pasteur concluded that the yeast cells growing on the surface of sugar solutions obtain their oxygen directly from the atmosphere. Growing below the surface they are deprived of this source of supply and meet their oxygen requirements by taking it directly from the sugar molecule, leaving the alcohol and carbon dioxid as by-products. It was subsequently shown by Buchner,[11] however, that an extract can be made of the yeast cells which added to sugar transforms it to alcohol and carbon dioxid. This reaction is now attributed to an enzyme in the yeast known as a zymase. Recent investigations of the fermentation of sugars by yeast extracts have demonstrated that zymase does not bring about the alcoholic fermentation of sugar by itself, but is dependent upon the presence of a co-enzyme (Harden[12]). This co-enzyme withstands the temperature of boiling water and is dialyzable. A phosphate is also indispensible, the breakdown of the sugar into alcohol and carbon dioxid being accompanied by the formation of a complex hexophosphate. The phosphate is split off from this compound and again rendered available for action by means of a special enzyme called hexophosphatase. Further enzymes are also present in the yeast cell by which the zymase and the co-enzyme are destroyed and an anti-enzyme which can directly check this destructive action. In the stages of the decomposition of the sugar pyruvic acid is produced, this being subsequently decomposed by a specific enzyme, carboxylase, into carbon dioxid and acetylaldehyd, the latter then undergoing enzymic reduction to ethyl alcohol. The action of yeast on sugars is thus much more complicated than originally supposed.

Higher sugars like lactose as well as the hexoses may be fermented with the production of alcohol and carbon dioxid. The alcoholic fermentation of milk is a common practice in the Orient for the production of alcoholic beverages. The lactose is first converted into dextrose and galactose by hydrolytic cleavage according to the following equation:

$$\underset{\text{Lactose.}}{C_{12}H_{22}O_{11}} + \underset{\text{Water.}}{H_2O} = \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Galactose.}}{C_6H_{12}O_6}$$

This cleavage is attributed to the presence of an enzyme, a "lactase." After the dextrose and galactose are once evolved they are attacked by the yeasts and alcohol and carbon dioxid produced. Both the Koumys of Southern Russia and the Kefir of the Caucasus are the products of alcoholic fermentation of milk.

The juices of fruits like apples and pears undergo alcoholic fermentation, the beverage being known as cider. Malt liquor is fermented to porter, beer, and ale. Fermented grape juice gives us wine and brandy, fermented molasses gives rum, fermented malted grain produces whisky. Commercial alcohol is made by the fermentation of the saccharose in molasses.

The alcoholic fermentation of sugars by bacteria may take place by changes similar to those now demonstrated for the yeast, but our information is not very complete on this point. Harden states that lactic acid and

acetic acid may be produced along with the alcohol according to the following equation:

$$\underset{\text{Hexose.}}{2C_6H_{12}O_6} + H_2O = \underset{\text{Lactic acid.}}{2C_3H_6O_3} + \underset{\text{Acetic acid.}}{C_2H_4O_2} + \underset{\text{Ethyl alcohol.}}{C_2H_5OH} + 2CO_2 + 2H_2$$

Kruse[13] suggests that lactic acid is not necessarily produced since there are other types of reaction possible. Thus acetic acid and alcohol may be derived from the hexose in this way:

$$\underset{\text{Hexose.}}{C_6H_{12}O_6} + H_2O = \underset{\text{Acetic acid.}}{C_2H_4O_2} + \underset{\text{Ethyl alcohol.}}{C_2H_5OH} + 2CO_2 + 2H_2$$

It has also been shown by Harden[14] that both *Bacillus coli* and *Bacillus typhosus* produce formic acid from dextrose. *Bacillus coli* elaborates an enzyme which splits up formic acid to carbon dioxid and water according to this equation:

$$2HCOOH + O_2 = 2CO_2 + 2H_2O$$

*Bacillus typhosus* has no action on formic acid, no $CO_2$ is elaborated and the formic acid remains in solution. This observation may explain the fact that there may be more acid in dextrose cultures of *Bacillus typhosus* than in dextrose cultures of *Bacillus coli.*

**Alcoholic Fermentation of Starch.**—Certain species of aspergillus like *Aspergillus oryzæ* and mucors like *Mucor Rouxi* convert starch into sugar, the action being attributed to an amylase or diastase. Under anaërobic conditions these organisms make a small amount of alcohol from the sugar.

**Lactic Acid Fermentation of Sugar.**—Sugar may be converted into lactic acid by yeasts and molds and this change is also effected by a number of bacterial species. It takes place theoretically according to the following equation:

$$\underset{\text{Dextrose.}}{C_6H_{12}O_6} = \underset{\text{Lactic acid.}}{2CH_3CHOHCOOH}$$

The conversion of the dextrose is ascribed to the action of an intracellular enzyme known as lactacidase, but no such enzyme has been isolated. Two kinds of lactic acid are known, the dextrorotatory and the levorotatory, and each kind comes from the activity of micro-organisms depending upon the species present and the temperature. Lactic acid is one of the principal substances arising in the fermentation of milk, cabbage, ensilage, etc. In milk it is a normal product, always appearing as the milk gets old, and it is to be attributed to the lactic acid bacteria or streptococci. The lactose is first inverted to dextrose and galactose, which are then converted into lactic acid.

**Butyric Acid Fermentation of Sugar.**—Many bacteria ferment the carbohydrates with the formation of butyric acid. This is done chiefly by anaërobes but a few aërobes also bring about this change. The reaction is supposed to take place as follows:

$$\underset{\text{Dextrose.}}{C_6H_{12}O_6} = \underset{\text{Butyric acid.}}{C_3H_7COOH} + 2CO_2 + 2H_2$$

The process is probably less simple than indicated by this equation. Butyric acid with its powerful and disagreeable odor is only one of the end products.

**Rarer Types of Sugar Fermentation.**—Other fermentations of sugar occur in which other substances are evolved such as citric acid, oxalic acid, formic acid, propionic acid, and valerianic acid. Citric acid is especially formed by certain molds like *Citromyces pfefferianus* and *Citromyces glaber*, oxalic acid by *Aspergillus niger*. Formic, propionic, and valerianic acids are usually formed in small quantities by the action of bacteria upon sugars in which acids like lactic and butyric are produced in abundance.

**Fermentation of Disaccharids.**—Probably only the monosaccharids, the hexoses, are fermented directly by the bacteria, the higher sugars being first inverted by enzyme activity to the simpler. Thus maltose is hydrolyzed to dextrose in the following manner:

$$\underset{\text{Maltose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose.}}{2C_6H_{12}O_6}$$

The process is apparently a hydrolytic cleavage, and has been ascribed to enzymes which have been named according to the sugars attacked, as maltase, lactase, etc. The reaction is probably more complicated than is indicated by the above equation. There is some evidence also that the higher sugars may be attacked directly with the production of acids without inversion to simple sugars. Thus it is said that saccharose is fermented by certain species of lactic acid bacilli without inversion to dextrose (Grigoroff[15]).

In some instances the ferment action is reversible. Thus maltose may be broken down to dextrose, but if dextrose be present in excess it is changed to maltose by some other ferment.

**Fermentation of Fats.**—Fats are fermented by many bacteria and by certain molds. This reaction is obscure and is ascribed to a fat-digesting or lipolytic enzyme known as lipase, which hydrolyzes the fat in the presence of moisture. The fats are converted into glycerin and the fatty acid corresponding to the original fat. According to Buchanan the hydrolysis of a fat may occur by a reaction of the following type:

$$\underset{\text{Stearin.}}{C_3H_5(C_{17}H_{35}COO)_3} + 3H_2O = \underset{\text{Glycerin.}}{C_3H_5(OH)_3} + \underset{\text{Stearic acid.}}{3C_{17}H_{35}COOH}$$

Palmitin and olein may be hydrolyzed in a similar manner. These fatty acids may be in turn attacked by bacteria and molds, usually in the presence of oxygen, with the production of butyric acid.

**Fermentation of Glycerin.**—Glycerin is fermented by several bacteria with the production of alcohol and formic acid. The reaction takes place according to this equation:

$$\underset{\text{Glycerin.}}{C_3H_5(OH)_3} = \underset{\text{Alcohol.}}{C_2H_5OH} + \underset{\text{Formic acid.}}{HCOOH}$$

It may also be fermented with the formation of butyric acid possibly by this reaction:

$$\underset{\text{Glycerin.}}{2C_3H_5(OH)_3} = \underset{\text{Butyric acid.}}{C_3H_7COOH} + 2CO_2 + 4H_2$$

The production of butyric acid from the glycerin formed originally from fats is an important cause of food decompositions, among which may be mentioned the rancidity of butter.

**Fermentation of Cellulose and Hemicellulose, etc.**—Cellulose and hemi-

cellulose, both of which contain carbohydrate radicles, may be attacked by several species of bacteria. There is probably an original hydrolysis of the polysaccharid after which the simpler sugars produced are directly attacked. Hemicellulose is utilized by a larger number of species than is cellulose. This fermentation is of great moment in the digestive processes of the herbivora. The digestive juices are unable to attack either cellulose or hemicellulose, the destruction of which is accomplished by the group of cellulose-splitting bacteria in the digestive tract.

Pectins, pectoses, and gums, compounds somewhat similar to cellulose, are also attacked by bacteria by an analogous process. These substances are hydrolyzed by many of the bacteria which produce diseases of plants, this being the first step in the invasion of the plant tissue by the organisms.

**Acetic Acid Fermentation.**—Acetic acid is formed by the bacteria in a number of fermentations, probably several different reactions taking place in its elaboration. The most important of these is the oxidation of the alcohol arising from the sugars through the action of the yeasts. The chemical change in this reaction may be represented as follows:

$$\underset{\text{Alcohol.}}{C_2H_5OH} + O_2 = \underset{\text{Acetic acid.}}{CH_3COOH} + H_2O$$

This type of oxidation is seen in the manufacture of vinegars from cider and is due to a large group of aërobic acetic acid bacteria which together make up what is called *Mycoderma aceti*, or mother of vinegar. The reaction is thought to be due to an intracellular enzyme, an alcohol oxidase, which has not thus far been isolated. With some of these acetic acid bacteria the reaction is involved. Thus *Bacterium Pasteurianum* found in beer produces gluconic acid ($CH_2OH(CHOH)_4COOH$) from dextrose, propionic acid ($C_2H_5$-$COOH$) from propyl alcohol ($C_3H_7OH$) and acetic acid ($CH_3COOH$) from ethyl alcohol ($C_2H_5OH$).

Acetic acid also arises as a kind of by-product in the fermentation of sugars by certain widely distributed bacteria like *Bacterium lactis aërogenes*, along with small quantities of alcohol, formic acid, etc. In certain complex anaërobic fermentations of carbohydrates acetic acid is likewise produced, but the steps in the process are not clearly known. According to Buchanan, the reaction may be of two types, one without gas represented by this formula:

$$\underset{\text{Dextrose.}}{C_6H_{12}O_6} = \underset{\text{Acetic acid.}}{3CH_3COOH}$$

and one with gas represented by this equation:

$$\underset{\text{Dextrose.}}{C_6H_{12}O_6} + 2H_2O = \underset{\text{Acetic acid.}}{2CH_3COOH} + 2CO_2 + 4H_2$$

**Fermentation of Starches.**—Starches, polysaccharids with the empirical formula ($C_6H_{10}O_5$) are attacked by many organisms, bacteria and molds. The starches are first converted into dextrins (erythrodextrin and achroödextrin) which are soluble in water by a hydrolytic extracellular enzyme, diastase, or amylase. These dextrins are then hydrolyzed to maltose and later to dextrose which is directly fermented by many types of bacteria.

Inulin, which has the same empirical formula as starch, is hydrolyzed to levulose instead of to dextrose, the levulose being likewise attacked by a number of bacteria.

## PUTREFACTION

Following Fischer we may define putrefaction as the decomposition of protein materials by micro-organisms, with the evolution of diverse split products. This is a slightly different significance from that employed by authors like von Hibler and Rettger who limit the term to a change in protein materials in which malodorous gases arise like those found in cadaveric decompositions, this being due almost entirely to the action of anaërobic bacteria. True putrefaction in the sense employed by von Hibler and Rettger may possibly be due also to certain types of aërobic bacteria and the presence or absence of odors may depend upon a variety of physical conditions. All types of albuminous materials can be attacked by micro-organisms and a number of substances may result from this action. Furthermore, the breaking down of proteins can be carried further by the action of micro-organisms than by the digestive juices alone. The most important decompositions occur with albumin, fibrin, and casein.

**Decomposition of Albumin.**—The decomposition of albumin by bacteria is primarily a simple process like the splitting of albumin by acids or alkalies. There is first a hydrolytic cleavage into albumoses (proteoses) and then into peptone. These complex molecules are then broken down in such a way that amino derivatives of the fatty series (amino-acids) and nitrogenous bodies of the aromatic series (skatol, indol) and sulphates (taurin) are produced. With these primary putrefactive products tryptophan is always present. The amino-acids are next converted to ammonia and fatty acids which are finally broken down to carbon dioxid, hydrogen, and methane. The important amino-acids are arginin, alanin, lysin, histidin, prolin, tryptophan, oxyprolin, isoleucin, cystin, leucin, glycocol, aspertic acid, tyrosin, valine, serin, glutamic acid, and phenylalanin. From the amino-acids polypeptids are elaborated.

In the case of some of the amino-acids special substances are produced. Thus leucin is converted to valerianic acid and the reaction is supposed to take place in accordance with the following equation:

$$\underset{\text{Leucin.}}{C_5H_{10}(NH_2)COOH} + 2H_2O = \underset{\text{Valerianic acid.}}{C_4H_9COOHNH_3} + CO_2 + 2H_2$$

Leucin is also decomposed in a similar fashion to caproic acid in part, and in larger part to butyric acid and acetic acid, while methane, carbon dioxid, and hydrogen are evolved in the process.

Glycocol and other amino-acids are split in much the same way. Tyrosin is likewise broken down to split products since it appears only in the early part of the putrefaction process.

**Native proteins** like egg-albumin, fibrin, and serum-albumin, are attacked by only a small number of bacteria, the most important of which is *Bacillus putrificus* of Bienstock. The solid protein material is liquefied by the organism, peptones, amino-acids, and related compounds being formed. An intense putrefactive odor is evolved from a mixture of gases including hydrogen sulphid ($H_2S$), and methyl mercaptan ($CH_3SH$). Butyric acid and ammonia also develop.

**Gelatin** is liquefied by a large number of bacteria but this liquefaction is evidently of two types. In some instances the gelatin apparently undergoes a simple hydrolytic cleavage, in consequence of which its power of solidification is lost. No protein degradation products are found. In other instances the gelatin is not only liquefied, but actually changed by the bacteria to simpler products like amino-acids, indol finally being present as

an end-product. The simple hydrolytic cleavage, as a result of which gelatin loses its capacity to solidify, might be differentiated as proteolysis from that type of gelatin liquefaction in which substances like indol or mercaptan are produced, indicating a true putrefaction.

Mavrojannis[16] finds two types of gelatin peptonization. In one case the change goes only so far as the formation of albumoses and the gelatin hardens in formaldehyd steam. In the other case the gelatin does not harden because it has been converted to fluid degradation products.

**Casein** is peptonized by many bacteria in much the same way that gelatin is attacked. According to Kalischer,[17] peptone, leucin, tyrosin, ammonia, and aromatic oxyacids are produced from casein by ferments of bacteria. The action is in general like that of trypsin. It is interesting to note that a rare amino-acid, diaminotrihydroxydodecanoic acid ($C_{12}H_{26}N_2O_5$) has been found by Fischer and Abderhalden as a product of the hydrolysis of casein.

**Hydrogen Sulphid.**—This substance is formed by a number of organisms from albumin and peptones, also from substances containing sulphur in easily reduced form (sulphates, thiosulphates). With albumin and peptone the hydrogen sulphid arises from a splitting of the protein molecule. Its presence may be determined by exposing a strip of filter paper moistened with a 10 per cent. solution of neutral lead acetate in the mouth of a flask in which the organisms are growing. Or the organisms may be cultivated on special media such as lead acetate agar. For accurate determinations of hydrogen sulphid production by bacteria, they should be cultivated in carefully prepared media, the gases given off being collected and analyzed for hydrogen sulphid by some quantitative method like that of Dupasquier. In this a measured quantity of gas is drawn through a standard solution of iodin containing some starch paste.

**Mercaptans and Sulphids.**—Among the end-products of protein decomposition are compounds containing hydrogen sulphid which is derived originally from the protein molecule. They include the organic hydrosulphids, which are usually called *mercaptans* because of their property of combining readily with mercuric oxid to form crystalline compounds, and the organic sulphids. The most important of such compounds are ethyl mercaptan, $C_2H_5SH$, and ethyl sulphid, $(C_2H_5)_2S$.

**Phenol** is produced from tyrosin with the intermediary products paraoxyphenyl-propionic acid, paraoxyphenyl-acetic acid, and paracresol. Phenols may be absorbed from the intestinal tract and appear in the urine as ethereal sulphates.

**Ptomains.**—In the previously discussed putrefactive processes in which indol, skatol, phenol, and paracresol are produced, the splitting off of the carboxyl group as carbon dioxid takes place after deaminization and the substances evolved are not highly toxic. If the carboxyl group is removed before the amino group is split off, highly toxic substances called amins are the result. These were formerly designated ptomains. The most important are ethylamin ($CH_2CH_2(NH_2)$) derived from alanin, phenylethylamin ($C_6H_4(OH)CH_2CH_2NH_2$) derived from tyrosin, and imidazoleëthylamin ($C_3H_3N_2CH_2CH_2NH_2$) commonly called histamin and derived from histidin. Cadaverin or pentamethyldiamin ($NH_2CH_2CH_2CH_2CH_2CH_2NH_2$) is similarly produced from lysin, an amino-acid, and tetramethylenediamin ($NH_2C_4H_8NH_2$) commonly called putrescin from the amino-acid ornithin. The most poisonous of all the ptomains is probably neurin ($(CH_3)_3C_2H_3$-NOH).

The total number of substances produced in protein decomposition is thus very large, the end-products including carbon dioxid, methane, hydro-

gen, nitrogen, hydrogen sulphid, ammonia, formic acid, acetic acid, butyric acid, valerianic acid, palmitic acid, acrylic acid, crotonic acid, glycollic acid, lactic acid, etc.

According to Salkowski the nitrogen-containing putrefactive products like indol, skatol, and skatol-carbonic acid arise from preformed mother substances in the protein molecule. Indol comes primarily from peptone. Even when nitrogenous materials like asparigin or urea are present indol does not appear unless peptone is likewise available. It is one of the final products of protein decomposition, has a sweetish disagreeable odor, and belongs to the aromatic series ($C_8H_7N$). Kendall[18] believes that skatol and indol can be formed by such bacteria as *Bacillus coli* and *Bacillus proteus vulgaris* from tryptophan, an amino-acid formed from protein. The tryptophan is converted to indol by the formation of a number of intermediary products, indolpropionic acid, indolacetic acid, and skatol. This is important since indol may be absorbed from the intestinal tract, oxidized in the liver to indoxyl and appear in the urine as indican.

Nitrogen-free aromatic substances homologous to benzoic acid, phenylacetic acid and phenylpropionic acid, are likewise evolved in protein decomposition. Baumann claims that these substances come from phenylamino-acids preformed in the protein molecule.

## ACTION OF BACTERIA ON PROTEINS AND CARBOHYDRATES

In the majority of instances in which bacteria multiply in nature, and also in our artificial cultures in the laboratory, both protein (nitrogenous) and carbohydrate (non-nitrogenous) materials are present and are utilized by the bacteria. The investigations of Wolfe and Harris and of Kendall and his co-workers have thrown much light upon the complex processes occurring when a mixture of these substances is present. In general the bacteria attack the utilizable carbohydrates first, obtaining their energy from their fermentation, and not attacking the protein molecule. By measuring the amount of ammonia in fluid cultures (deaminization), it is possible to determine the extent to which the protein is attacked in the presence and absence of carbohydrates. Thus much larger quantities of ammonia are produced by *Bacillus coli* in dextrose-free broth than in broth containing dextrose, indicating a greater utilization of the protein. Similar measurements have been made for a large number of pathogenic organisms. When utilizable carbohydrates are present, indol and skatol are not produced by cultures of *Bacillus coli*, etc., because these organisms attack the carbohydrates first and the acid produced is sufficient to inhibit their growth before the protein is attacked. As was originally pointed out by Durham[19] and more recently investigated by Harden,[20] Clark and Lubs,[21] and others, many organisms like *Bacterium lactis aërogenes* and *Bacillus cloacæ* are able to withstand the acid produced by their fermentation of the sugars. They continue to multiply, attacking now the protein and producing ammonia and other alkaline substances. The final reaction in media containing these organisms is thus *alkaline*. Such organisms are called methyl red negative, as distinguished from the methyl red positive organisms like *Bacilus coli*, *Bacillus typhosus*, etc. They usually give a positive Voges-Proskauer reaction due to the formation of acetylmethylcarbinol.

## ENZYMES

The changes which take place in fermentation and putrefaction are ascribed to the activities of *enzymes* which are secreted by the bacteria and

which bring about a chemical rearrangement of the molecules. Many of the enzymes have been obtained free from the bacterial cell and have been carefully investigated. Their presence in bacterial and yeast cultures has been demonstrated with many species, and in other species which bring about chemical changes it is assumed that the enzymes are present inside the cells and are liberated as the cells die or from the living cells. Enzymes are usually regarded as a special kind of protein which acts as a catalytic agent, taking part in the reactions, but not being used up in the process. They may enter into definite combinations with the substances acted upon and be liberated later. Enzymes are characterized by reversibility of reaction. Thus maltase which splits maltose to dextrose according to this reaction:

$$\underset{\text{Maltose.}}{C_{12}H_{22}O_{11}} + H_2O + \text{maltase} = \underset{\text{Dextrose.}}{2C_6H_{12}O_6} + \text{maltase}$$

really never converts all the dextrose to maltose but brings about an equilibrium between the amounts of maltose and dextrose in the solution. Some dextrose is always left. If now fresh dextrose be added the amount of maltose will be increased, indicating a conversion of this added dextrose to maltose by the enzyme which was first operative in converting the maltose to dextrose (a reversal of the reaction).

Enzymes may be classed as intracellular and extracellular. The extracellular are found free in the culture media while the intracellular are inside the cell. This distinction is largely artificial since conceivably the intracellular enzyme may be liberated from the cell and act upon the various substances about it or remain in the cell and act upon substances after they are taken up.

Enzymes act in various ways, by hydrolyzing, by oxidizing, and by splitting in other methods than hydrolytic. Reduction is also often regarded as an enzyme activity. Little is known of the chemical composition of enzymes and they are defined by certain of their properties. Thus enzymes are colloids; have optimum, maximum, and minimum temperatures at which their activities are exercised; are destroyed at temperatures above 70° C. and by boiling; have no action at zero degrees and are not destroyed by drying. They are relatively unstable; deteriorating rapidly or slowly after isolation; are destroyed by light, especially by the violet and ultraviolet rays; are affected by chemicals in various ways.

In many instances mother substances, called pro-enzymes or zymogens are supposed to be present, acting as the precursers of enzymes.

## CLASSIFICATION OF ENZYMES

The best classification has been suggested by Oppenheimer[22] and by Fuhrman[23] who divide enzymes into A. hydrolases, B. oxidases, C. catalases and D. zymases.

**Hydrolases.**—The hydrolases bring about their changes by the addition of water, the substances at the same time being split into simpler products. This is seen in the inversion of cane-sugar by invertase, the reaction taking place possibly by the following equation:

$$\underset{\text{Saccharose.}}{C_{12}H_{22}O_{11}} + H_2O + \text{invertase} = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Fructose.}}{C_6H_{12}O_6} + \text{invertase}$$

The hydrolases are further subdivided into four main groups, the ester-

ases or lipases, the carbohydrases, the amidases and proteases, and the coagulases.

*Esterases or Lipases.*—These enzymes act upon the esters and fats and the most important are those which decompose fats into fatty acid and glycerin. Thus the stearin in butter is hydrolyzed by certain micro-organisms with the production of stearic acid and glycerin according to this reaction:

$$\underset{\text{Stearin.}}{(C_{17}H_{35}CO_2)_3C_3H_5} + 3H_2O + \text{lipase} = \underset{\text{Stearic acid.}}{3C_{17}H_{35}COOH} + \underset{\text{Glycerin.}}{C_3H_5(OH)_3} + \text{lipase}$$

Lipases are rarely produced by ordinary micro-organisms, one of the most interesting in this connection being the hog-cholera bacillus (Welch).

*Carbohydrases.*—It is usually believed that only the simpler sugars, the hexoses, are attacked by micro-organisms (yeasts, molds, and bacteria), the higher sugars being reduced to the lower by a process of hydrolysis due to enzymes. This is certainly true for the majority of organisms. It is significant however that some of the lactic acid organisms, streptococci and micrococci, described by Grigoroff are supposed to ferment saccharose without any corresponding action upon dextrose. In general the higher sugars are first inverted to the lower before utilization by bacteria. Maltose is inverted to dextrose by maltase. Saccharose or cane-sugar is inverted to dextrose and fructose by invertase or sucrase. Lactose is inverted to dextrose and levulose by lactase.

Polysaccharids with the general formula, $(C_6H_{10}O_5)n$, are hydrolized by different enzymes. Thus starch is decomposed by diastase or amylase first into dextrins and then into maltose which in turn is attacked by maltase and then fermented. Cellulose, a carbohydrate, is attacked by the enzyme cellulase or cytase. Inulin, a carbohydrate, is attacked by inulase and hydrolyzed to levulose while the hemicelluloses are hydrolyzed by the ferment seminase. Pectin, found in the middle lamella separating the cells of plants, is hydrolyzed by pectinase. This reaction is of importance in the flax industry and in certain plant diseases such as black rot of the cabbage and rot of the carrot.

*Amidases and Proteases.*—The enzymes which act upon amins, proteins, and related compounds are known as amidases and proteases. They may be divided into amidases, ereptases, nucleases, trypsins, and pepsins. Of the amidases the most important is urease which converts urea into ammonium carbonate by this reaction:

$$\underset{\text{Urea.}}{(NH_2)_2CO} + 2H_2O + \text{urease} = \underset{\text{Ammonium carbonate.}}{(NH_4)_2CO_3} + \text{urease}$$

The ereptases or erepsins are enzymes which decompose proteoses and peptones to amino-acids and ammonia. The peptins decompose proteins into proteoses and peptones. The trypsins also decompose proteins, breaking them down into amino-acids and ammonia. Such enzymes convert the solid proteins, curd in coagulated milk, gelatin, egg-albumin, and blood-serum into fluid or liquid products.

*Coagulases.*—The coagulation of milk by rennin or lab-ferment is the most common reaction due to enzymes produced by bacteria of this group. The caseinogen is converted to casein which may be subsequently liquefied by the same bacteria, when they produce trypsin or erepsin. Similar enzymes are thrombase which causes the coagulation of blood and myosinase which converts the myosinogen of the muscle into myosin in rigor mortis.

These last two enzymes are not produced by bacteria, but a reaction similar to the coagulation of blood in the body is sometimes produced by such organisms as the pneumococcus in fluid blood-serum.

**Oxidases.**—The oxidases are those enzymes which bring about changes in organic matter by the addition of oxygen to the molecule. The best understood is the oxidation of ethyl alcohol to acetic acid by some of the acetic acid bacteria (*Bacterium aceticum*). This may take place by the following scheme:

$$\underset{\text{Ethyl alcohol.}}{C_2H_5OH} + O_2 = \underset{\text{Acetic acid.}}{CH_3COOH} + H_2O$$

Similar oxidases produced by bacteria oxidize the sugars to acetic acid and citric acid.

**Catalases** decompose hydrogen peroxid into oxygen and water and are widely distributed in nature. They are not produced by bacteria.

**Zymases.**—The zymases are those enzymes which bring about decomposition of organic compounds without hydrolysis or oxidation, seeming merely to split up the compounds into simpler compounds. Hence they are often called splitting enzymes. They are usually intracellular. Zymase is produced by yeast cells, by molds, and by bacteria. It converts glycose into ethyl alcohol and carbon dioxid according to this reaction:

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} + \text{zymase} = 2\underset{\text{Ethyl alcohol.}}{C_2H_5OH} + 2CO_2 + \text{zymase}$$

As already indicated, several other enzymes take part in the fermentation of glucose to ethyl alcohol and carbon dioxid. Another enzyme, lactacidase, converts glucose into lactic acid thus:

$$\underset{\text{Glucose.}}{C_6H_{12}O_6} + \text{lactacidase} = 2\underset{\text{Lactic acid.}}{CH_3—CHOH—COOH} + \text{lactacidase}$$


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## Chapter V

# METHODS OF CULTIVATING BACTERIA

### PURPOSES OF MEDIA

BACTERIA may be cultivated upon a great number of artificial media which are designed to serve several specific purposes.

1. To provide a menstruum in which micro-organisms may be plated or distributed uniformly when the menstruum is fluid and fixed in position when this is solidified. Ordinary cooking gelatin and agar, a Japanese seaweed, are the important substances which meet this requirement. The melting-point of gelatin is about 28° C. and this medium serves for the plating of organisms which grow at temperatures below this. The melting-point of agar is about 46° C. and agar serves for the plating of organisms which grow at the temperature of the body, 37° C., and below this. Both agar and gelatin provide a matrix to which various substances may be added which supply nutritive materials for the growth of bacteria. Gelatin is itself a protein compound and is utilized by the bacteria in their development. Agar is probably a pectin, but so far as we know does not furnish any nutritive materials for micro-organisms.

2. To supply nutritive materials by means of which bacteria may be brought to cultivation. With many species this is comparatively simple since they are not particular in their requirements but grow in both vegetable and animal proteins in various combinations, provided that the content in salts, acids, and alkalies is not too high. Other species are fastidious, growing only in media containing special substances or on special combinations of materials. The gonococcus can be brought to cultivation only when animal protein is present, preferably of human origin. The tubercle bacillus is equally exacting in its requirements, often requiring special combinations containing animal protein. With certain bacteria like the leprosy bacillus the particular artificial medium which will bring it to cultivation has not thus far been found.

3. To supply substances of exact chemical composition upon which bacteria exercise a specific action by means of which we may separate the species and varieties. Such substances as the sugars, glucosids, and alcohols, for instance, are utilized by particular species of bacteria and we may classify and identify them by means of these reactions.

4. To provide a menstruum for the growth of bacteria simulating as closely as possible the substances in nature in which bacteria develop. Thus the nitrifying bacteria of the soil may be cultivated upon certain nitrogenous combinations which are not unlike those actually met with in the soil.

To meet these purposes hundreds of varieties of artificial media have been devised from time to time and of these a large number have proved of permanent value to the bacteriologist and are widely employed. The particular methods of preparing these media differ in different laboratories, these differences relating chiefly to the proportion of the various ingredients and to the reaction of the final product. The reaction of the medium may be determined by titration with phenolphthalein or by means of hydrogen-ion estimations.

## DETERMINATION OF REACTION

**Titration with Phenolphthalein.**—Put 5 c.c. of the medium to be tested in a white porcelain dish and add 45 c.c. of distilled water. Boil briskly for one minute. Add 1 c.c. of phenolphthalein solution (5 grams of commercial phenolphthalein to 1 liter of 50 per cent. alcohol). Titrate immediately with N/20 solution of sodium hydrate. The end point is marked by a faint but distinct pink reaction. Estimate the amount of normal sodium hydrate or normal hydrochloric acid solution required to bring the entire lot of medium up to the desired acidity or alkalinity. This is expressed in terms of acidity or alkalinity according to Fuller's scale in which alkaline media are signified by the minus sign and acid media by the plus sign. (Thus +1 media have an acid reaction of 1 per cent., −1 media have an alkaline reaction of 1 per cent., estimated by normal acid or alkaline solutions.)

*Eyre's Scale.*[1]—There action or titre of media is here expressed by indicating the number of cubic centimeters of normal alkali or normal acid that would be required to render 1 liter of the medium exactly neutral to phenolphthalein. The sign + (plus) is prefixed to this number if the original solution reacts acid, and the sign − (minus) if it reacts alkaline. For example, "meat extract + 10" indicates a sample of meat extract which reacts acid to phenolphthalein, and which would require the addition of 10 c.c. of normal NaOH per liter to neutralize it.

**Titration by the Estimation of the Hydrogen-ion Concentration.**—In this method the reaction of the medium is determined by the addition of indicators to small samples and the amount of acid or alkali found which on addition will bring the reaction of the final product to the desired point. There are a number of indicators which may be employed, but in general it is only necessary to use a few, such as phenol red, cresol red, bromthymol blue, or bromcresol purple. In carrying out this titration 5 c.c. of the medium is added to 5 c.c. of distilled water and then a few drops of the indicator. In the case of phenol red (phenolsulphonephthalein) the indicator is prepared by dissolving 0.04 grams in 30 c.c. of alcohol and diluting it to 100 c.c. with distilled water. A standard solution of NaOH is now added drop by drop till the sample of medium containing the phenol red shows a slight, but distinct pink color. The standard solution of NaOH is made by accurately titrating an N/10 solution of the alkali against an N/1 solution of succinic acid with phenolphthalein as an indicator of the end point. It is usually used in dilutions of 1 : 10, 1 : 20, 1 : 40. The color changes in the sample are best determined by comparing them with standard indicators in solutions of known acidity and alkalinity. The amount of the standard NaOH solution which must be added to the entire lot of medium to give the reaction is now estimated.

## METHODS OF OBTAINING PURE CULTURES OF BACTERIA

Strains of bacteria of which all the cells have approximately the same morphology and the same physiological reactions are called *pure cultures.* Theoretically all pure cultures are supposed to originate from a single bacterial cell, but special methods of obtaining strains from single cells have recently been developed and these are now usually called *pure-line strains* or *single-cell strains.* Various procedures may be carried out in order to obtain pure cultures and pure line strains of which the most important are the following:

1. **Plating.**—Bacteria may be separated into groups, each of which usually comes from a single bacterial cell, by the "poured plate" method of

Koch. In carrying out this method the bacteria are first distributed uniformly in some substances like gelatin or agar which remains fluid at temperatures which are so low that the bacteria put in them are not killed and becomes solid at temperatures suitable for the bacterial cells to multiply and form individual colonies. Melted gelatin remains fluid at temperatures above 25° C. and solidifies below this. It is therefore available for bacteria that develop well at the temperature of the room, about 22° C., as, for example, the water bacteria. Melted agar remains fluid at temperatures above 42° C. and solidifies at temperatures below this. Agar, therefore, serves for plating bacteria which grow well at the heat of the body, about 37.5° C. Since all the pathogenic bacteria which invade the tissues and produce disease in man grow at the body temperature agar is the most valuable medium for them. In general small quantities of the organisms to be plated are first emulsified in broth or peptone solution and 3 drops of this emulsion carried over to a tube of melted gelatin or agar at about 43° to 44° C. These drops are obtained by the use of a small loop of platinum or iridium and are known as oesas. From the first tube of gelatin or agar three oesas are carried to a second tube and again three oesas from the second to a third. All three tubes are now poured into Petri plates and after solidification are incubated at the proper temperature. The individual cells are fixed in place in the gelatin or agar, find in the medium proper materials for their nourishment, and develop into characteristic colonies which appear in twenty-four to forty-eight hours. Frequently the bacteria are emulsified in the first tube of melted agar instead of in broth or peptone solution and sometimes only one or two oesas are carried over to the second and third tubes of medium. For very accurate work the platinum needles are made in uniform sizes each of which contains a quantity of fluid which can be measured accurately. There are many minor variations in the procedures followed by different bacteriologists in carrying out Koch's poured plate method. The basic principle upon which the method is founded remains the same, however.

In many instances the colonies which develop on gelatin and on agar are the progeny of single cells. In some cases, as in plates from milk, the colonies are derived from groups of cells which are present in the milk in closely agglomerated particles. Frequently bacteria stick to each other so persistently, by virtue of their viscid surfaces, that satisfactory emulsions are difficult to obtain. In such cases the bacterial masses must be shaken repeatedly, by hand or in a shaking machine, before the masses of organisms are broken up into separate cells.

2. **Streaking.**—Isolated colonies may be obtained by smearing the surface of solid media like agar, blood agar, or solidified blood-serum with the material containing the organisms. The medium is first melted and poured into large Petri plates. The material is then distributed uniformly over the surface, usually by the use of a sterile glass rod. The colonies which develop are frequently but not always the progeny of single cells. This method is especially valuable for the examination of the body fluids, blood, or urine, and for the study of fecal material.

3. **Rapid Transfer.**—With a few organisms pure cultures can be obtained by repeated rapid transfer of the growth from one tube of a favorable fluid medium to another. The principle of this method lies in the fact that the organisms are supplied with a medium which is peculiarly favorable to them but either indifferent to or unfavorable to the growth of other species. The organisms supplied with their suitable conditions for multiplication rapidly outgrow the others. Sometimes pure cultures are obtained after a

few transfers. Pure cultures of lactic acid bacteria often result from frequent transfer of material from one milk tube to another or from one flask of milk to another. The lactic acid bacteria find favorable conditions for multiplication in milk and rapidly outgrow other organisms. Certain anaërobes may also be obtained pure by this method. *Bacillus Welchii* multiplies rapidly in milk and after half a dozen transfers from actively growing cultures, usually at the end of ten to twelve hours, pure cultures of the "gas bacillus" result.

4. **Selective Media.**—Pure cultures may often be obtained by the use of media which contain substances, especially favorable to the development of certain species but which do not support the life of other bacteria. This method is especially valuable for the isolation of some of the true soil bacteria. Certain types of soil bacteria grow upon silica gel containing a mixture of inorganic salts. This medium does not permit the development of other organisms and pure cultures of the soil bacteria may be obtained after one or two transfers. In other cases media are utilized which favor the development of some species and inhibit or restrain other types. The medium devised by von Drigalski and Conradi favors the multiplication of typhoid and paratyphoid bacilli and inhibits the growth of *Bacillus coli* and other intestinal forms. The typhoid and paratyphoid bacilli may thus be obtained from the dejecta by this method, while they practically never appear on plates made from the dejecta by the use of ordinary agar or gelatin. In much the same way Loeffler's blood-serum enhances the growth of the diphtheria bacillus, colonies of which appear in twelve to fifteen hours in this medium before other organisms present in the exudate in the throat have had time to develop into colonies.

5. **Veillon's Method.**—Pure cultures can frequently be obtained by transferring colonies from deep agar shake cultures. The original material is transferred to some transparent medium like agar or gelatin, this medium is then shaken to ensure a uniform distribution of the organisms and incubated at a suitable temperature. After two to three days isolated colonies appear in the depths of the medium. These colonies are now "fished" and transferred to other media by the use of sterile pipets. The shake cultures should be examined with a hand lens to determine the presence of suitable colonies. The block of agar may be removed from the tube and broken up to liberate the colonies, the surface of the agar being sterilized by some disinfectant solution like the tincture of iodin. This method is especially available for the isolation of anaërobic bacteria.

6. **Cantu's Method.**—Pure cultures of certain organisms like *Proteus vulgaris* and *Proteus mirabilis* may be obtained by inoculating the water of condensation which collects at the bottom of agar tubes. The bacteria multiply in this water and then spread up on the surface of the agar, the *proteus* spreading more rapidly than other bacteria. Transfers are now made from the growth on the agar near the bottom of the tube to the water of condensation of fresh agar tubes. After a few transfers pure cultures of the *proteus* result.

7. **Pure-line Strains.**—To obtain strains of bacteria which are definitely derived from single cells isolated bacteria must be selected and transferred to media suitable for their development. The best method to accomplish this is the pipet method devised by Barber. Here a few drops of fluid, 4 or 5, are placed in a coverslip and examined under a high dry or an immersion lens. A few bacteria are introduced into one of the drops and some of this transferred by a capillary pipet to another drop. If the number of organisms is sufficiently small separate bacteria may be transferred by pipets

to fresh drops. The drops containing single organisms are now carried by pipets to fluid media. The entire procedure can be observed under the microscope. By the use of Barber's method strains of organisms can be obtained which are surely the progeny of a single cell, so-called pure-line strains.

### INOCULATION OR SEEDING OF CULTURE-MEDIA

In order to maintain the growth of bacteria and study their cultural reactions, that is, the effects which bacteria produce in the substances surrounding them, the media must be inoculated or seeded with small quantities. This inoculation or "transfer" is usually carried out with needles of platinum or iridium, the ends of which may be bent into loops (oesas) or left straight. Platinum loops are usually employed for inoculating the surface of solid media or fluid media and straight needles for carrying bacteria to the depths of solid media like agar or gelatin. Considerable skill is necessary for the successful transfer of organisms which grow delicately like the streptococci and pneumococci. With solid media the material must be smeared gently on the surface. With fluid media the organisms should be rubbed with some of the fluid against the wall of the tube and then allowed to flow gently into the material below. Frequently successful transfers may be accomplished by inoculating the water of condensation first, then smearing the surface of the solid medium with the emulsion so prepared.

### INCUBATION OF CULTURES

In order that bacteria may have the heat necessary for their multiplication, cultures must be kept at constant temperatures. For the ordinary pathogenic bacteria which grow best at or near 37.5° C., the temperature of the body, incubators or thermostats are most commonly employed. These are warmed by gas or electricity and various thermoregulatory devices are utilized to secure constancy of heat. When large numbers of bacteria are investigated, especially when instruction is given to students, incubator rooms of special construction are more suitable than individual incubators. For water bacteria and certain of the soil organisms which grow best in gelatin at lower temperatures, about 22° C., the heat of the ordinary room in winter is satisfactory for their cultivation. In summer the temperature of the room is frequently above the melting-point of gelatin and special incubators must be employed. For bacteria which grow at high temperatures, 50° to 70° C., incubators must be expressly adjusted to maintain these high degrees of heat.

### AËROBIC METHODS FOR CULTIVATING BACTERIA

For bacteria which grow aërobically, that is, in an atmosphere of oxygen, a free supply of this substance is essential. With plates a sufficient amount of oxygen passes through the spaces between the bottom plates and the covers. Flasks and tubes should be plugged with cotton through which oxygen diffuses rapidly. In general they should not be sealed tightly. The percentage of oxygen in the atmosphere is so high that the amount necessary for the growth of aërobic bacteria is always provided when the atmosphere has free access to the cultures.

### ANAËROBIC METHODS FOR CULTIVATING BACTERIA

For bacteria which grow only in the complete or partial absence of atmospheric oxygen special methods must be employed to produce anaërobic conditions. Many anaërobes grow in the depths of solid media like

agar or gelatin. The media should be freshly prepared or boiled just before inoculation to drive off the absorbed oxygen and after inoculation may be covered with another layer of sterile medium. Anaërobes often develop in the depths of liquid media covered with some substance impervious to the passage of oxygen such as liquid vaselin. Reducing substances like methylene-blue, which indicate the absorption of oxygen by changing to a leuco-base, may also be added to the tubes before sealing with the vaselin. Anaërobic conditions are often produced in the bottom of flasks of fluid media in which aërobic bacteria are growing at or near the surface, the oxygen being used up and not penetrating to the depths. In general special methods must be employed to drive out the oxygen from the cultures or to cause its absorption. The cultures may be placed in glass jars such as those devised by Novy and the air driven out by some gas which has no injurious action upon bacteria. The best gas for this purpose is hydrogen which may be generated in a Kipp apparatus. Illuminating gas may be used but this often contains substances which are injurious to certain types of organisms. After the air has been driven out the jars should be sealed hermetically.

Several methods have been devised for the absorption of the atmospheric oxygen as a result of which practically only nitrogen is left behind. This is inert as far as the growth of most bacteria is concerned. The simplest method is the use of pyrogallic acid and a strong solution of caustic soda or caustic potash, usually 25 per cent. The combination of the pyrogallic acid and the alkali makes a strong absorbing mixture which removes the major portion of the oxygen. The cultures should be placed in receptacles, closed, and the two substances allowed to come in contact. The pyrogallic acid method may be used for plates in a Novy jar or for culture tubes. For the latter the cotton plugs may be saturated with the mixture of acid and alkali, as in the scheme devised by Wright, and the tubes sealed hermetically. The small amount of oxygen in the culture tubes is absorbed by the mixture and many anaërobes grow well below.

The oxygen may also be drawn out of the cultures mechanically, a vacuum or partial vacuum being produced. In carrying out this method the tubes are usually placed in a desiccator and the air drawn off by suction. Frequently a combination of the vacuum method and the pyrogallic acid method is employed, part of the air being removed by suction, the oxygen present in the remainder being absorbed by the pyrogallic acid and caustic potash. This method is valuable for micro-organisms which are very sensitive to small amounts of atmospheric oxygen.

**McIntosh and Fildes' Method for Cultivating Anaërobes.**—One of the best methods for cultivating anaërobes is that devised by McIntosh and Fildes[2] and this has been modified by a number of bacteriologists (Smillie,[3] Brown[4]). In this method the oxygen is removed by the oxidation of hydrogen through the medium of palladinized asbestos wool. A piece of asbestos wool is impregnated with platinum or palladium black and suspended in an air-tight jar of glass or metal provided with an inlet for hydrogen. A stream of hydrogen is then introduced which combines with the atmospheric oxygen to form water. The palladium asbestos is made by adding 1.5 c.c. of a 10 per cent. solution of palladium chlorid to 5.25 grams of asbestos wool in a small evaporating dish. The wool is molded into a flat mass about 1 inch square and gently heated to dryness. The addition of a little concentrated HCl may be necessary for the solution of the palladium chlorid. The impregnated wool is then heated to reduce the palladium chlorid, first in a smoky gas flame till it is covered with a layer of carbon, then in a blow-

pipe. The palladium asbestos will now light a jet of hydrogen which impinges on it. The impregnated asbestos is placed in a receptacle of copper gauze, heated over a flame till red hot and suspended to the lid of the jar containing the cultures; this is then hermetically sealed, preferably with plastocene. Hydrogen is now introduced very slowly either from a cylinder of liquid hydrogen or from a Kipp apparatus. This hydrogen now unites with the oxygen, forming a film of water on the sides of the tubes. Eventually a negative pressure develops inside the jar. The hydrogen should not be allowed to enter too rapidly since large quantities may give rise to an explosion.

**Partial Oxygen Tension.**—Many aërobic organisms grow well in an atmosphere where the oxygen is reduced below the amount present in the air. The air is best removed by the production of a partial vacuum by suction. The same method suffices for the cultivation of anaërobes which tolerate small quantities of oxygen. The growth of strongly aërobic species like *Bacillus subtilis* also removes a certain amount of oxygen from closed tubes and creates a lowered oxygen tension. This is favorable to the growth of several organisms like the gonococcus and the bacillus of bovine abortion.

## STUDY OF PURE CULTURES

**Observation of Cultural Reactions.**—The cultural reactions of bacteria, *i. e.*, the changes which their growth produces in culture-media, are observed usually on the first, second, and third days and then after a somewhat prolonged period, ten to fifteen days. The size and shape of colonies, the mass of growth, the pigment produced, the turbidity resulting in clear fluid media, the changes in reaction of milk, its coagulation or failure to coagulate, etc., are all points of importance in the identification of species. Colonies should be observed with the naked eye and always with a low magnification produced by a hand-lens or by the lower powers of the ordinary microscope. Their size on various days should be measured exactly, preferably in millimeters. Liquefaction of solid media like gelatin or blood-serum should be carefully watched for since proteolysis is an important differential criterion, particularly with the anaërobes.

**Indol Production.**—The production of indol by bacteria is usually tested in cultures two to three days old in ordinary broth, peptone solution, or sugar-free broth. Various tests may be employed. The best are Salkowski's, Ehrlich's, or the vanillin test of Steensma.[5] In the Salkowski test a few drops of concentrated $H_2SO_4$ are added to the culture tubes. If indol has been produced a pink color forms in the presence of nitrites. With bacteria which do not reduce nitrates to nitrites it is necessary to add a few drops of a dilute nitrite solution in order to demonstrate the indol. In the Ehrlich test 1 c.c. of a 2 per cent. solution of paradimethylaminobenzaldehyd in 95 per cent. alcohol is added to the culture, after which concentrated HCl is added drop by drop. When indol is present a red zone appears between the culture and the reagent. This red zone deepens on standing. The color may be extracted with chloroform. The vanillin test is carried out by the addition of 5 drops of a 5 per cent. vanillin solution in 95 per cent. alcohol and 2 c.c. of concentrated $H_2SO_4$ or HCl to 5 c.c. of the culture. An orange color develops in two to three minutes in the presence of indol. A reddish-violet color is produced by tryptophan.

**Nitrites.**—The reduction of nitrates to nitrites by bacteria is best determined in cultures of the organisms in nitrate broth or nitrate agar. Two solutions are used in testing.

A. Sulphanilic acid, 8 grams, in dilute HCl (1 : 20), 1000 c.c.

B. A naphthylamin hydrochlorid, 5 grams, in dilute $H_2SO_4$ (1 : 125), 1000 c.c.

Two or 3 drops of each reagent are added to the tube of nitrate broth or allowed to flow down the surface of a nitrate agar slant. If nitrites are present the broth takes on a uniform red color. With agar a deep red zone develops at the base of the slant.

**Phenol.**—The presence of phenol is best determined in flasks containing 50 to 100 c.c. of plain broth. After three to four days incubation of the culture at 37° C., 5 c.c. of concentrated HCl are added and the material distilled. To 10 to 20 c.c. of the distillate 0.5 c.c. of Millon's reagent (mercurous nitrate in nitric acid) are added. A red color develops in the presence of phenol. On the addition of 0.5 c.c. of a ferric chlorid solution a violet color is produced if phenol is present.

**Acid or Alkali Production.**—The presence of acids or alkalies in cultures is determined by the addition of various indicators to neutral media before inoculation. The best indicators are litmus, neutral red, China blue or Andrade. The Andrade indicator consists of 100 c.c. of a 0.5 per cent. aqueous solution of acid fuchsin to which 16 c.c. of normal NaOH have been added. The production of acids or alkalies by the growth of the organisms is indicated by the change in the color of the indicators. Or the indicators may be added to cultures of the organisms of various ages and the presence of acids or alkalies determined at definite periods of growth.

A better method of determining acid or alkali production is to estimate the hydrogen-ion concentration in cultures at a definite period. The hydrogen-ion concentration of the medium should be carefully estimated before inoculation, the cultures incubated at constant temperatures and the reaction determined in the regular way by comparison with color standards.

**Gas Formation.**—The production of gases by bacteria is tested by the cultivation of the organisms in deep solid media or in fermentation tubes of broth containing fermentable substances. In the solid media gas bubbles collect in the depths especially about the growth. For broth cultures the Smith fermentation tube is most frequently employed. Here gas collects in the closed arm. A more economic apparatus is known as the Durham fermentation tube. This consists of a tube of small caliber inverted in a tube of larger caliber. The small tubes are automatically filled by the fluid during sterilization. For the determination of the kind of gases produced the Smith tube and not the Durham must be used. The presence of carbon dioxid is roughly indicated by adding concentrated NaOH or KOH by which it is absorbed. If the remainder of the gas is explosive it is usually regarded as hydrogen. For an accurate determination of the gases produced by bacteria they must be collected in quantity and analyzed chemically.

**Hydrogen Sulphid** ($H_2S$).—Hydrogen sulphid is best determined by the cultivation of organisms in alkaline media containing 0.1 c.c. of 1 per cent. solution of ferric tartrate or lead acetate. A yellowish precipitate is formed in the bottom of the culture tubes on the addition of these substances to the media. This precipitate turns black when $H_2S$ is produced by the organisms. The reaction is best carried out by the cultivation of the organisms in strong peptone solution.

**Methyl Red Reaction.**—Organisms which produce acid in standard solutions of carbohydrate (Clark and Lubs) give positive methyl red reactions at the end of a definite period. These organisms are known as methyl

red positive. Organisms failing to give the methyl red reaction at the same period are known as methyl red negative.

**Voges-Proskauer Reaction.**—This reaction is determined by cultivating the organisms in standard solutions for definite periods, three to five days, and adding 1 c.c. of a 50 per cent. solution of KOH. After twenty-four hours at room temperature a pink color develops at the top of the tube. This color is due to the presence of acetyl-methyl-carbinol.

## ANTAGONISM AND SYMBIOSIS IN CULTURES

In general we study bacteria in the laboratory in pure culture, that is, in strains derived from single colonies on plates or obtained from single cells by such methods as Barber's. In nature single species of organisms are formed only under exceptional circumstances as in infections of man and animals, or in fermenting material where chemical substances favor the multiplication of certain types which rapidly overgrow and crowd out the others. Thus in sour milk one finds sometimes practically a pure culture of lactic acid streptococci. Frequently in nature many different species of bacteria multiply side by side and the observed changes are by no means always to be ascribed to the activity of single species.

In cultures different types of bacteria may be present and multiply at the same time and this phenomenon is sometimes spoken of as a kind of *symbiosis*. Thus anaërobic bacteria may be brought to cultivation at the bottom of broth in flasks in which aërobic bacteria are also multiplying, as first pointed out by Theobald Smith. Here the aërobes apparently exhaust the supply of oxygen to such a degree that proper conditions are produced for the development of anaërobes. Sometimes the changes found in culture media are to be attributed to the growth of different species at the same time. Thus in mixed cultures of streptococci and paratyphoids the streptococci may first invert the lactose to hexoses which are in turn attacked by the paratyphoids with gas production. Thus we may have an apparent fermentation of lactose by organisms which have no action upon this sugar under normal circumstances.

In many instances the culture media contain substances which are *antagonistic* to the growth of certain species and favorable to that of others. The diphtheria bacillus grows rapidly on Loeffler's blood-serum while staphylococci and streptococci are first inhibited, appearing only some hours after the Klebs-Loeffler organisms. Similarly the multiplication of *Bacillus coli* and other intestinal bacteria is inhibited on Drigalski-Conradi medium while *Bacillus typhosus* finds in it substances which are favorable to its development. The original reaction of the media is of considerable moment for the growth of organisms. Thus the cholera vibrio multiplies rapidly in highly alkaline gelatin and soon overgrows other micro-organisms.

Bacteria sometimes exhibit an antagonistic action to the multiplication of other organisms and filtrates of the media in which one species has grown are found unsuitable for the growth of others. This effect is frequently to be attributed to changes in reaction or to an accumulation of deleterious products of growth. If the reaction is adjusted or the media cooked to destroy labile decomposition products it will support the growth of the organisms without difficulty (Felton[6]). In other instances the nutritive materials in the media are actually used up by one species and the supply is not enough for the other. In addition to this kind of antagonism which finds its explanation in the character of the changes produced in the media there are many instances where one species seems to *inhibit* the

growth of others and we have no adequate explanation for the reaction. Thus *Staphylococcus aureus* seems to inhibit the growth of the anthrax bacillus, but not that of *Bacillus pyocyaneus*.

*Bacillus pyocyaneus* hinders the development of the gonococcus. Certain organisms are especially sensitive to the presence of other organisms in cultures. Bitter[7] has pointed out that the plague bacillus will not grow in mixed cultures with streptococci even if heavy inoculations are made. Noguchi[8] has recently shown that the slightest bacterial contamination of the cultures prevents the development of *Leptospira icteroides*. Conversely certain species of micro-organisms seem to have a *favorable* influence upon others. Staphylococci favor the growth of the influenza bacillus which develops giant colonies on plates containing both species. Davis[9] interprets the production of large colonies as a satellite phenomenon and a specific reaction of the influenza bacillus toward bacteria, yeast, fungi, and plant and animal tissues. It is due to diffusable products which stimulate the growth of the organism in connection with hematin or hemoglobin. These substances are destroyed by heating to 120° C. in the autoclave.

Finally certain organisms multiply freely in the presence of others and one cannot detect any favorable or unfavorable action of the one upon the other. Thus staphylococci and streptococci grow well together and the gonococcus is readily brought to cultivation in the presence of pyogenic cocci.

## DETERMINATION OF NUMBERS OF BACTERIA

**Counting Bacteria.**—The number of viable bacteria in any given fluid material may be roughly determined by making agar or gelatin plates and counting the colonies which develop at the end of different periods of time. Only those plates which show uniformly distributed colonies should be counted. In order to obtain such plates it is often necessary to dilute the original material. The number of colonies on the plates should then be multiplied by the factor of dilution. For the estimation of the number of colonies on each plate examination with the naked eye may be supplemented by examination with a hand-lens to determine whether actual colonies are present or merely specks of débris. In all reports the magnification afforded by the hand lens should be stated since lenses of high magnification often bring into view colonies which are not visible with lenses of low power.

*Counting Bacteria in Blood and Exudates.*—The number of bacteria in blood may be obtained on agar plates incubated preferably at 37° C. Since the organisms which invade the blood are somewhat fastidious in their culture requirements special media containing whole blood or blood-serum give better results than ordinary media. For the cultivation of special organisms like the gonococcus from pus it is always necessary to employ some medium like hydrocele agar upon which this organism will grow. For pus, exudates, urine, etc., plates should be made with ordinary media. Blood for cultures should be obtained from the veins, preferably from one of the branches of the femoral veins. The skin should be carefully sterilized first, the blood withdrawn by means of a sterile needle and syringe. Blood may also be obtained by puncturing the lobe of the ear and collecting a few drops in sterile tubes. This method is not reliable since the organisms normally present in the deeper layers of the skin may be accidentally introduced into the cultures. In the examination of all materials of this description specimens should be carefully studied by bacterioscopical methods to determine the presence of organisms.

*Counting Bacteria in Feces.*—The number of bacteria in fecal material may be determined by counting the colonies on agar or gelatin plates. For the fermenting acid-forming bacteria plates of lactose litmus agar or v. Drigalski-Conradi medium are preferable. Only a fraction of the intestinal bacteria is obtained by these methods since many of the fecal bacteria do not grow on ordinary media or are inhibited by the more vigorous *Bacillus coli*. For the aciduric bacteria special media with an acidity equal to 50 c.c. normal acid to the liter should be used. This acidity inhibits the ordinary intestinal bacteria and the aciduric species grow readily. For the determination of obligate anaërobes, anaërobic methods should be employed but the results are unsatisfactory since many of the intestinal bacteria are facultative anaërobes and grow well under anaërobic conditions. Spore-bearing bacteria may be obtained by heating samples of fecal material to a temperature which suffices to destroy vegetative bacteria and making plates from this heated material. These plates should be incubated both aërobically and anaërobically. Frequently obligate anaërobes may be thus obtained. It should be remembered that cultures from such heated material represent only the spore material in the feces.

This spore material may or may not represent bacteria vegetating in the intestinal contents.

1. Eyre: Bacteriological Technique. 2. ed. 1915, p. 153.
2. McIntosh and Fildes: Lancet, 1916, i, 768.
3. Smillie: J. Exper. M., 1917, xxvi, 59–66.
4. Brown: J. Exper. M., 1921, xxxiii, 677–681.
5. Steensma: Ztschr. f. physiol. Chem., 1906, xlvii, 25. *See also:* Nelson: J. Biol. Chem., 1906, xxiv, 527.
6. Felton: Johns Hopkins Hosp. Bull., 1923, xxiv, 313.
7. Bitter: Report of the Egyptian Plague Commission. Cairo, 1897.
8. Noguchi: Monogr. Rockefeller Inst. M. Research, No. 20, 1924, August 9.
9. Davis: J. Infect. Dis., 1921, xxix, 178.

## COMPOSITION OF THE USUAL MEDIA

Fluid media are usually made with some animal protein material as a base, such as meat infusion prepared from the lean muscles of beef, sheep, swine, or veal. Commercial meat extracts like Liebig's extract of beef may also be employed. Different substances are added to the meat infusion or meat extract, particularly salt, peptone, sugars, alcohols, and glucosids. The peptone may be prepared in the laboratory but commercial products like Witte's peptone are more available. Commercial peptone has no definite composition but usually contains a mixture of albumoses. Infusions of vegetable proteins may likewise be used as a base for fluid media. The methods of preparing the most important fluid media and their composition follow:

**Meat Infusion Broth.**—Strip 1 pound of lean meat (beef or veal) free from fat or tendons; grind or chop finely, soak over night in 1000 c.c. water. Filter through cheese-cloth, bring to the boiling-point over a slow fire and filter through filter paper. Add water to bring the volume back to 1000 c.c. and add:

| | |
|---|---|
| Witte's peptone, | 10 grams |
| Sodium chlorid, | 5 grams |

Neutralize if necessary.

Tube and sterilize this in the autoclave for five minutes at 15 pounds pressure.

**Meat Extract Broth:**

| | |
|---|---|
| Liebig's meat extract, | 2.5 grams |
| Witte's peptone, | 10 grams |
| Sodium chlorid, | 5 grams |

Tube and sterilize five minutes in the autoclave at 15 pounds pressure.

**Sugar-free Broth.**—In order to rid the meat infusion broth of fermentable sugars which obscure the fermentation reactions with pure sugars, prepare meat infusion broth in the ordinary way; after boiling and filtering inoculate with a fluid culture of *Bacillus coli* and let stand over night, either at room temperature or at 37° C. Boil five minutes, filter through filter paper, and add 10 grams of peptone and 5 grams of sodium chlorid.

Pour in a flask and sterilize five minutes in the autoclave at 15 pounds pressure.

**Sugar Broth.**—Add 1 to 2 per cent. glucose (dextrose) to neutral meat infusion or meat extract broth and use in Smith or Durham fermentation tubes. The sugar which may be present in meat infusion or rarely in meat extract is a monosaccharid (muscle sugar? glucose?) and does not interfere. Sterilize either by steam for thirty minutes for three consecutive days or in the autoclave about ten minutes at 5 pounds pressure. Other monosaccharids like galactose and levulose may be prepared in the same way. For higher sugars, disaccharids and polysaccharids, it is necessary to use sugar-free media and sugars free from dextrose. These sugars are inverted to monosaccharids by heat and great care should be taken in sterilization. They may be sterilized by steaming thirty minutes on three consecutive days or in the autoclave ten minutes at 5 pounds pressure. For very careful work a better method is to prepare sugar-free broth, and sterilize it before adding the sugars. The appropriate amount of the sugar to be used may be added from stock 10 per cent. solutions which remain sterile by virtue of the bactericidal action of strong sugar solutions.

**Glycerin Broth:**

| | |
|---|---|
| Meat infusion broth, neutral or slightly acid, | 1000 c.c. |
| Glycerin, C. P., | 30–50 c.c. |

Sterilize by steaming in the Arnold one-half hour on three consecutive days. For the tubercle bacillus adjust reaction to +1 acid to phenolphthalein. Sugar-free broth may also be used as the stock solution.

**Dunham's Peptone Solution:**

| | |
|---|---|
| Witte's peptone, | 10 grams |
| Sodium chlorid, | 5 grams |
| Water, | 1000 c.c. |

Dissolve the peptone and salt in the water, boil five minutes, filter through filter paper till clear; tube and sterilize five minutes in the autoclave at 15 pounds pressure.

**Acetic Acid Broth:**

| | |
|---|---|
| Dextrose, | 10 grams |
| Acetic acid, | 20 c.c. |
| Broth, | 1000 c.c. |

Tube and sterilize in autoclave ten to fifteen minutes at 5 pounds pressure.

Kendall recommends the addition of sufficient glacial acetic acid to a liter of 2 per cent. glucose broth to make the reaction equal to 50 c.c. of normal acid. A precipitate forms which settles out and leaves a clear supernatant fluid.

Kendall: Bacteriology, General, Pathological, and Intestinal. 2. ed. 1921, p. 202.

**van Ermengem's Broth:**

| | |
|---|---|
| Sodium chlorid, | 5 grams |
| Witte's peptone, | 10 grams |
| Dextrose, | 20 grams |
| Lean beef, | 1000 grams |
| Water, | 1 liter |

Tube and sterilize in autoclave ten to fifteen minutes at 5 pounds pressure.

**Ox Bile Broth:**

| | |
|---|---|
| Ox bile, | 10 c.c. |
| Lactose, | 10 grams |
| Peptone, | 10 grams |
| Water, | 1000 c.c. |

**Smith's Tissue Broth.**—Add a small piece of the kidney or the liver of a rabbit or guinea-pig to neutral broth and use in the Smith fermentation tube. The bits of tissue should be removed aseptically from an animal immediately after killing.

**Beef Liver Broth.**—Boil 500 grams of beef liver in 1000 c.c. of water two hours and filter. Add 10 grams of peptone and 1 gram dipotassium phosphate. Adjust reaction to neutral.

**Blood Broth.**—A few drops of blood may be added to ordinary broth to enhance the growth of pathogenic bacteria. Human or rabbit blood is preferable, and the blood of horses, beef, and sheep is also valuable.

**Park and Williams' Trypsin Broth.**—Soak over night 300 to 500 grams of meat in a liter of water to which 0.4 per cent. sodium carbonate has been added. Heat to 80° C., cool to 38° C., and add 15 c.c. of liquid trypsin. Keep at 38° C. five hours, stirring frequently, adding 10 c.c. toluol or crystals of thymol if material is kept over night. Add normal HCl to neutralize, boil seven minutes, strain, and establish desired reaction. Boil one-half hour, filter, and sterilize.

**Nitrate Solution:**

| | |
|---|---|
| Peptone, | 1 gram |
| Potassium nitrate, | 0.2 gram |
| Distilled water, | 1000 c.c. |

Tube.

Sterilize in the Arnold one-half hour on three consecutive days.

**Martin's Peptone Solution.**—Clean a number of pig stomachs free from fat and mince finely. Mix:

| | |
|---|---|
| Minced stomachs, | 200 grams |
| Hydrochloric acid (pure), | 10 grams |
| Water (at 50° C.), | 1000 c.c. |

Keep at 50° C. twenty to twenty-four hours in glass or porcelain vessel, not enamel, to prevent the peptone coming in contact with metal. Heat to 80° C. to stop digestion and filter through a layer of absorbent cotton. Heat filtrate to 70° C., neutralize to litmus, sterilize in autoclave at 10 pounds pressure for fifteen to twenty minutes.

**Martin's Peptone Broth.**—Mix equal quantities of meat infusion and Martin's peptone after neutralization, keeping peptone and broth at 70° C. Titrate at room temperature and establish desired reaction. Autoclave for fifteen minutes to clear, titrate, and set reaction again. Filter through paper and cotton and sterilize in autoclave at 15 pounds pressure for one-half hour.

**Martin's 0.2 per cent. Glucose Broth:**

| | |
|---|---|
| Martin's peptone broth, | 1000 c.c. |
| Glucose, | 2 grams |

**Hottinger's Digested Meat Infusion Broth** (Modified by Park, Williams, and Krumwiede).—Free 1500 grams of meat from fascia and cut into pieces the size of the finger. Drop them piece by piece into 1500 c.c. boiling water, stirring constantly. Boil thoroughly. Remove meat and pass through chopping machine. Cool water to 37° C. and add sodium bicarbonate, 1.5 grams to liter. Put 550 grams of chopped meat in 2 liter Erlenmeyer flasks, add the water up to neck of flasks, keeping temperature at 37° C. To each flask add 3 grams pancreatin, 10 c.c. chloroform and 10 c.c. toluol. Cork tightly, shake well, and incubate at 37° C. over night. Next day shake and add more pancreatin unless meat has begun to digest and fluid is yellow in color. Continue digestion four to five days at room temperature or two to three days at 37° C. until meat is digested completely and gives off an offensive odor. If alkaline, acidify with small amount of HCl. According to Hottinger this stock can be diluted ten to twenty times and used as desired. Diluted one half with water (1 part stock broth, 1 part water) it serves as an excellent medium for ordinary micro-organisms except the diphtheria bacillus.

Park and Williams state that the stock does not keep well and after neutralization with HCl should be diluted with equal quantities of water, shaken, decanted and filtered through cheese-cloth. It is then boiled for a few minutes, filtered through absorbent cotton and paper till clear, and autoclaved for one-half hour at 15 pounds pressure.

**Hiss's Calcium Carbonate Broth.**—Add 1 gram powdered calcium carbonate and 1 gram glucose to 100 c.c. meat infusion broth. Sterilize the dry calcium carbonate in hot air chamber before using.

Bolduan employs small pieces of marble instead of calcium carbonate. These media are valuable for obtaining mass cultures of the pneumococcus or the streptococcus.

**Hiss's Serum-water Media.**—Dialyze the serum to remove salts, dilute with two to three times its volume of distilled water, tube and sterilize at 100° C. The removal of the salts and the dilution prevents coagulation. For fermentation tests add some indicator and the appropriate sugar. Acid fermentation is indicated by the change in reaction and the resulting coagulation, gas production by bubbles in the coagulum.

**Holman's Serum Broth.**—Add 100 c.c. fresh serum to 300 c.c. distilled water. Mix this with the following medium:

| | |
|---|---|
| Peptone, | 40 grams |
| Liebig's meat extract, | 12 grams |
| Sodium chlorid, | 20 grams |
| Distilled water, | 1000 c.c. |

Make mixture neutral to phenolphthalein and add 4 grams of the carbohydrate to be employed and 4 grams of Andrade's indicator.

The serum may also be filtered before use if necessary.

Holman: J. Infect. Dis., 1914, xv, 209.

**Plain Milk.**—Take ordinary market milk, neutralize if necessary and tube in 10 to 12 c.c. quantities. Sterilize in the autoclave fifteen minutes at 5 to 10 pounds pressure. The cream may be left in the milk or the skimmed milk used.

**Litmus Milk.**—Add about 100 c.c. of litmus tincture to 1000 c.c. milk or enough to give the milk a purple lilac color. Tube and sterilize in the autoclave five minutes at 15 pounds pressure. The milk may be rendered fat free by skimming off the cream or may be used with the cream in which case a small portion rises to the top in each tube.

**Litmus Milk from Milk Powder:**

| | |
|---|---|
| Skimmed milk powder, | 52.7 parts |
| Azolitmin, | 1 part |

Grind the azolitmin with the milk powder in a mortar. Dissolve the mixture in distilled water. Add 1 part of the mixture to 9.5 parts of water. Sterilize in the Arnold thirty minutes or in the autoclave at 15 pounds pressure for five minutes.

**Kahn's Brom Cresol Purple Milk.**—Partially skimmed milk, with the reaction adjusted to *p*H 7.2, is tubed in 10 c.c. quantities and autoclaved. Prior to tubing, 40 c.c. of brom cresol purple indicator are added to each liter of milk.

Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.

**Petruschky's Litmus Whey.**—Warm fresh milk, coagulate the casein with very dilute hydrochloric acid, and filter off the whey. Neutralize the whey with dilute sodium carbonate solution. Steam one to two hours to precipitate any acid albumin produced by the acid. Filter. A clear colorless neutral fluid containing chiefly lactose is thus produced. Add solution of litmus to give the proper color.

Muir and Ritchie recommend making the medium slightly alkaline with sodium carbonate after the precipitation of the casein and then boiling. The medium is then filtered, neutralized, tubed, and sterilized.

**Durham's Modification of Petruschky's Milk Whey.**—Gently warm fresh milk and clot with rennin. Strain off the whey through muslin. Cautiously neutralize the whey with 4 per cent. citric acid solution, using neutral litmus solution as an indicator. Heat on water-bath to 100° C. half an hour to coagulate the proteins. Filter through paper or Berkefeld candles and add litmus solution till the whey is distinctly purple. No mineral acids should be used. The finished whey contains no protein, only water, lactose, the salts of the milk and traces of a monosaccharid, dextrose or galactose.

**Gordon's Nutrose Medium:**

| | |
|---|---|
| Ascitic fluid, | 15 c.c. |
| Distilled water, | 35 c.c. |
| Nutrose, | 1 gram |

Boil and filter. Add 1 part of this to 2 parts ordinary peptone agar. Steam half an hour and tube.

**Clark and Lub's Solution:**

| | |
|---|---|
| Witte's peptone, | 5 grams |
| Dextrose, | 5 grams |
| Dipotassium hydrogen phosphate, | 5 grams |
| Distilled water, | 1000 c.c. |

Filter tube and sterilize thirty minutes in the Arnold or five minutes in the autoclave at 15 pounds pressure.

**Voges-Proskauer Solution:**

| | |
|---|---|
| Witte's peptone, | 10 grams |
| Glucose, | 10 grams |
| Sodium chlorid, | 5 grams |
| Distilled water, | 1000 c.c. |

Filter, tube, and sterilize in the Arnold thirty minutes or in the autoclave five minutes at 15 pounds pressure.

**Solid media** are made with some jelly-like substance like gelatin agar or blood-serum as a base, primarily to give consistency to the medium. Agar or agar-agar is prepared from a seaweed known usually as Chinese or Ceylon moss which grows abundantly in Japan. It is frequently called

gelose. Agar contains a number of chemical substances. For bacteriological purposes it is thoroughly washed and the active substance left is usually regarded as a pectin or a hemicellulose. It yields large amounts of galactose on hydrolysis with dilute acids (10 per cent.), and also yields other sugars as pentoses and some hexose other than galactose. These sugars are held in firm combination and are not acted upon by bacteria growing in agar. It is generally believed that agar does not furnish any nutritive substance for the growth of bacteria. Gelatin is an animal protein, prepared from connective tissues, cartilage, ligaments, and tendons. It usually contains about 18.3 per cent. nitrogen, 0.6 per cent. sulphur, 50 per cent. carbon, 7 per cent. hydrogen, and 23 per cent. oxygen. It is utilized by bacteria in their growth and from it amino-acids, indol, and other substances are produced. The coagulated serum of many animals is also used to give consistency to media, usually the serum of horses, beef, sheep, and rabbits. Human serum is valuable for certain fastidious microorganisms. The chemical composition of the serum varies among the different species but the protein constituents of all types are utilized, various decomposition products resulting, peptone, amino-acids, indol, etc. Other protein materials which coagulate on heating like egg-albumen may be used. Different substances are added to agar, gelatin, serum or coagulated egg, and many varieties of solid media have been devised by different bacteriologists. Some of the most important are prepared as follows:

**Meat Infusion Gelatin:**

| | |
|---|---|
| Gelatin, | 120 grams |
| Peptone, | 10 grams |
| Sodium chlorid, | 5 grams |
| Meat infusion, | 1000 c.c. |

Dissolve the ingredients in the meat infusion by bringing slowly to the boiling point, tube, sterilize in the autoclave five minutes at 10 pounds pressure.

**Meat Extract Gelatin:**

| | |
|---|---|
| Gelatin, | 120 grams |
| Peptone, | 10 grams |
| Sodium chlorid, | 5 grams |
| Meat extract, | 2 grams |
| Water, | 1000 c.c. |

Dissolve the peptone, salt, and meat extract in the water by bringing them gently to a boil. Add the gelatin, maintaining at the boiling point till the gelatin is completely dissolved.

Tube, sterilize in the autoclave five minutes at 10 pounds pressure.

**Sugar Gelatin.**—Prepare ordinary meat infusion or meat extract gelatin and add 1 to 2 per cent. glucose. For other sugars make the gelatin from sugar-free broth or sugar-free meat extract and sterilize by steaming thirty minutes three consecutive days or in the autoclave ten minutes at 5 pounds pressure.

**Meat Infusion Agar (gelose):**

| | |
|---|---|
| Meat infusion, | 1000 c.c. |
| Peptone, | 10 grams |
| Sodium chlorid, | 5 grams |
| Agar, | 15 grams |

Add the salt and the peptone to the meat infusion, heat over the gas stove, add the agar after finely shredding it and bring slowly to a boil, holding at this temperature till all the ingredients are dissolved. Neutralize by the addition of 5 per cent. NaOH solution, using phenolphthalein paper as an indicator. Cool to 60° C., add two eggs beaten up in about 150 c.c.

water, replace on the gas stove over a low fire, bring slowly to a boil, and maintain at this temperature eight to ten minutes or until the egg is thoroughly coagulated. Bring volume up to 1000 c.c. by the addition of water. Strain and filter through moist filter paper or through wet absorbent cotton. Tube and sterilize five minutes in the autoclave at 15 pounds pressure.

**Meat Extract Agar:**

| | |
|---|---|
| Meat extract, | 1000 c.c. |
| Peptone, | 10 grams |
| Sodium chlorid, | 5 grams |
| Agar, | 2 grams |

Add the salt and peptone to the water, heat to the boiling point, add the finely shredded agar and keep at the boiling point till the agar is dissolved. Neutralize in the usual way, clear with egg, bring volume up to 1000 c.c., filter, tube, and sterilize five minutes in the autoclave at 15 pounds pressure.

**Sugar agar** should be prepared from agar made of meat infusion or meat extract freed from sugar by fermentation with *Bacillus coli*. The sugars may be added directly in proper amounts, $\frac{1}{2}$, 1, or 2 per cent., and the agar then sterilized by steaming one-half hour three consecutive days or by holding five minutes in the autoclave at 5 to 10 pounds pressure. The sugar may also be kept in concentrated solution, about 10 per cent., which remains sterile, and appropriate amounts added to sterile agar. This method avoids the subsequent sterilization which may break down some of the higher sugars to dextrose.

**Glycerin Agar.**—Add 5 to 8 per cent. glycerin (C. P.) to meat infusion agar. Make it +1 acid to phenolphthalein if to be used for tubercle bacillus.

**Tissue Agar.**—Add a small portion of the liver or kidney of a guinea-pig or rabbit to melted agar, which is then allowed to solidify. The tissues should be removed aseptically from freshly killed animals.

**Beef Liver Agar:**

| | |
|---|---|
| Ground beef liver, | 1 part |
| Water, | 4 parts |
| Peptone, | 1.5 per cent. |
| Sodium chlorid, | 0.5 per cent. |
| Agar, | 2 per cent. |

Make faintly alkaline to litmus. Tube and sterilize.

**Hottinger's Agar.**—Dilute peptone broth with water (1 part of broth to 1 part of water) and add agar, 1.5 grams to 1000 c.c. This agar works well with the usual laboratory organisms except the diphtheria bacillus and the gonococcus.

**Pfeiffer's Blood Agar.**—Allow a few drops of sterile rabbit's blood or blood from other animals to flow over the surface of slant agar, or add a few drops of the blood to melted agar tubes held at 40° to 42° C. These are then slanted and allowed to cool.

Pfeiffer: Ztschr. f. Hyg., 1893, xiii, 357.

**Ascitic Fluid Agar.**—Add 3 to 5 c.c. sterile ascitic fluid to melted agar, hold at 40° to 42° C., slant and allow to solidify. Incubate at 37° C. to determine whether the medium is sterile.

**Hydrocele Fluid Agar.**—Add 3 to 5 c.c. sterile hydrocele fluid to melted agar held at 40° to 42° C. Slant the agar tubes and allow to solidify. Incubate at 37° C. to determine if the medium is sterile.

**Salt Agar.**—Add 2 to 5 per cent. sodium chlorid (C. P.) to ordinary agar.

Especially recommended for the plague bacillus and for luminescent bacteria.

**Herring Salt Agar.**—Prepare broth from fresh herring instead of beef and add 2 per cent. ordinary salt or sea salt. Valuable for luminescent bacteria.

**MacConkey's Bile Salts Agar:**

| | |
|---|---|
| Sodium glycocholate, | 5 grams |
| Peptone, | 1.5 grams |
| Lactose, | 3.5 grams |
| Agar, | 1.5 grams |
| Tap water, | 100 c.c. |

Dissolve agar and peptone in the water, clear in the usual manner and add the lactose and sodium glycocholate just before using.

Organisms producing acid from lactose precipitate the bile salts.

**Neutral Red Glucose Agar.**—Prepare meat infusion or meat juice agar in the ordinary way. Add 1 to 2 per cent. glucose and 1 per cent. neutral red indicator. Sterilize in the autoclave ten minutes at 5 pounds pressure. Other sugars may be added to the agar in which case the meat infusion or meat extract should be made sugar-free by the fermentation with *Bacillus coli* and the medium sterilized by steaming thirty minutes on three consecutive days or by heating ten to fifteen minutes in the autoclave at 5 pounds pressure.

**Huntoon's "Hormone" Agar:**

| | |
|---|---|
| Chopped beef heart or muscle, | 500 grams |
| Peptone (bacto), | 10 grams |
| Agar, | 16 grams |
| Sodium chlorid, | 5 grams |
| Egg, | 1 gram |
| Water, | 1000 c.c. |

Mix ingredients but do not filter through cloth, filter paper or cotton.

For streptococci add 0.15 per cent. dextrose and enough laked blood to give the medium a light purple tint.

Huntoon: J. Infect. Dis., 1918, xxiii, 169.

**Hall's Testicular Infusion Agar.**—1. Mix 500 grams ground beef testicle with 1000 c.c. distilled water.

2. Soak over night at room temperature.

3. Heat to 50° C.; keep warm one hour in thermostat at 37° C.

4. Boil, strain, and restore to 1000 c.c. with distilled water.

5. Add 2 per cent. peptone (Witte's or Difco), 3 per cent. agar, 0.5 per cent. glucose, and 0.3 per cent. $NaH_2PO_4$.

6. Soak one hour.

7. Melt in autoclave at 10 pounds pressure for twenty minutes.

8. Titrate with phenolphthalein and add N/1 NaOH to neutralize.

9. Repeat titration. Five c.c. should require 1 to 2 c.c. N/20 NaOH to display color.

10. Tube, sterilize by autoclaving at 10 pounds pressure for twenty minutes.

Hall: J. Bact., 1916, i, 343.

**Gessard's Agar:**

| | |
|---|---|
| Agar, | 50 grams |
| Peptone, | 20 grams |
| Glycerin, | 50 c.c. |
| Water, | 1000 c.c. |

Neutralize and sterilize at a low temperature.

**Veillon's Agar:**

| | |
|---|---|
| Agar, | 10 grams |
| Dextrose, | 10 grams |
| Potassium nitrate, | 1 gram |

Neutralize and sterilize in the Arnold thirty minutes or in the autoclave ten to fifteen minutes at 5 pounds pressure.

**Kligler's Lead Acetate Agar:**

| | |
|---|---|
| Nutrient agar (0.5 per cent.), | 1000 c.c. |
| Lead acetate, | 0.5–1 gram |
| Glucose, | 2 grams |

Kligler: Am. J. Pub. Health, 1917, vii, 805.

Lead acetate agar can also be made by adding to beef-extract containing 30 grams peptone, 5 c.c. of a 0.1 per cent. basic lead acetate solution per liter. The agar should have a reaction of *p*H 7.2 to 7.6.

Organisms producing hydrogen sulphid blacken lead acetate agar when grown on it.

Fellers, Shostrom, and Clark J. Bact., 1924, ix, 235.

**Holman's Triple Indicator Agar.**—Add to ordinary agar with a reaction of *p*H 7.2:

| | Per cent. |
|---|---|
| Lactose, | 1 |
| Saccharose, | 1 |
| Dextrose, | 1 |
| Andrade indicator, | 1 |
| Brom thymol blue (sat. aq. sol.), | 2 |
| Lead acetate (1 per cent. aqueous), | 6 |

Tube, sterilize in the autoclave at 10 pounds pressure for fifteen minutes and slant in a deep bath.

**Eberson's Yeast Agar.**—Macerate 10 grams of baker's or brewer's yeast in 100 c.c. of water for twenty minutes, steam for two hours with the temperature not exceeding 100° C. Clarify by adding Merck's dialyzed iron (5 per cent. ferric oxid) and filtering through glass wool. Add equal amounts of this prepared yeast to 1 per cent. agar containing 2 per cent. peptone and 0.4 per cent. potassium phosphate. Adjust the reaction to *p*H 7.6 which after heating is reduced to 7.4. Tube in 10 c.c. amounts and autoclave for one-half hour at 15 pounds pressure. This medium is especially valuable for the preservation of delicate organisms like the meningococcus, gonococcus, influenza bacillus, and the pertussis bacillus.

Burky has modified Eberson's medium by growing a laboratory strain of yeast in broth containing 2 per cent. peptone, 2 per cent. maltose, 0.5 per cent. sodium chlorid in shallow layers in Erlenmeyer flasks. The reaction of the broth is not adjusted. After forty-eight hours the yeast growth is steamed for one hour over a water-bath and centrifugalized till the supernatant fluid is clear. One part of the clear supernatant fluid is added to 1 part of 1 per cent. agar solution containing 0.4 per cent. disodium hydrogen phosphate.

Eberson: J. Am. M. Ass., 1919, lxxii, 852.
Burky: J. Am. M. Ass., 1824, lxxxiii, 999.

**Hitchens' Low Percentage Agar.**—This medium is made from a meat infusion base prepared by adding 1 kilo of ground lean beef to 1 liter of water and incubating at 37° C. for forty-eight hours. The juice is strained

off, heated over a water-bath to boiling and again strained. Peptone is now added, 40 grams, making 2 per cent. in the final product and $KNO_3$, 4 grams, making 0.2 per cent. After the peptone is dissolved, the reaction is adjusted to *p*H 7.5. It is then filtered and autoclaved at 15 pounds pressure for thirty minutes. Enough glucose is added to make 0.2 per cent. in the final medium. Agar jelly is prepared by drying thread agar thoroughly, washing in running water over night, and adding enough distilled water to make a 6 per cent. solution. The agar is dissolved in the autoclave, the reaction adjusted to *p*H 7.5, and the product then cleared by straining through cotton and gauze. This agar solution is now added to the fluid base in quantities sufficient to make up a 0.1 per cent. agar medium, both being heated before mixing. It is finally autoclaved at 15 pounds pressure for twenty minutes.

Hitchens: J. Infect. Dis., 1921, xxix, 390.

**Koch's Inspissated Blood-serum.**—Obtain blood-serum, horse, sheep, or ox, as nearly sterile as possible, and tube in 10 c.c. quantities. Sterilize by heating to 57° C. for an hour on eight successive days. Inspissate (*i. e.*, harden) by heating to 65° C. slowly and maintaining at that temperature several hours.

**Coagulated Blood-serum.**—Obtain serum of beef, horse, or sheep, and tube in 10 c.c. quantities. Coagulate in a slanting position and sterilize by steaming on three successive days or in the autoclave thirty minutes at 15 pounds pressure. For stab cultures the media may be solidified and sterilized in the upright position.

**Loeffler's Blood-serum:**

| | |
|---|---|
| Fresh ox serum, | 3 parts |
| Glucose broth, 1.5 glucose, | 1 part |

Tube, sterilize in a Koch coagulator by boiling the water five minutes on three consecutive days. The tubes may also be sterilized by placing them in a horizontal autoclave, steaming for a short time till the serum is coagulated, and then keeping them five minutes at 15 pounds pressure.

**Potato.**—Clean good-sized potatoes carefully, cut into slabs ½ inch thick, wash in running water over night, place in Petri plates, and sterilize five minutes in the autoclave at 15 pounds pressure. The potatoes may also be cut into cylinders with a potato borer somewhat smaller than a test-tube, divided into half cylinders, placed in test-tubes, and sterilized five minutes in the autoclave at 15 pounds pressure.

**Glycerin Potato.**—Prepare potato in the usual way and soak two to three hours in 10 : 25 solution of glycerin. Tube and leave a few cubic centimeters of glycerin in the bottom of each tube. Sterilize by steaming thirty minutes on three consecutive days. This does not alter the glycerin. Glycerin potato is especially recommended for the tubercle bacillus.

## SPECIAL MEDIA

### SPECIAL MEDIA FOR THE GONOCOCCUS

A number of media have been devised for the cultivation of the gonococcus which is somewhat fastidious in its requirements. The best results are obtained by the use of protein materials from the human body, blood-serum, hydrocele fluid or ascitic fluid. The following media may be recommended:

**Guarnieri's Gelatin Agar Mixture:**

| | |
|---|---|
| Sodium chlorid, | 5 grams |
| Peptone, | 25–30 grams |
| Gelatin, | 40–60 grams |
| Agar, | 3–4 grams |
| Water, | 50 c.c. |
| Meat infusion, | 950 c.c. |

**Welch's Modification of Guarnieri's Medium:**

| | |
|---|---|
| Agar, | 5 grams |
| Gelatin, | 80 grams |
| Sodium chlorid, | 5 grams |
| Peptone, | 10 grams |
| Meat infusion, | 1000 c.c. |

**Wertheim's Gonococcus Medium:**

| | |
|---|---|
| Agar, | 2 parts |
| Sterile human serum, | 1 part |

The agar is prepared in the ordinary way and just before use each tube is melted and cooled to 40° C. at which temperature the serum is added.

**Gurd's Blood Agar.**—Prepare 2 per cent. agar with an acid reaction +6 to phenolphthalein. Melt the tubes, cool to 40° C., and add 5 drops of sterile defibrinated human blood to each 5 c.c. of agar.

**Vedder's Starch Medium.**—Add 500 grams of beef to 1000 c.c. water, extract in the ice-chest over night, boil, and strain. Add 1.5 per cent. agar, dissolve and bring reaction 0.2 to 0.7 per cent. + acid to phenolphthalein. Cool and clear with egg. Add 1 per cent. starch, heat in Arnold to dissolve, shaking several times. Tube and autoclave fifteen minutes at 5 pounds pressure.

Good for meningococcus and gonococcus.

Vedder: J. Infect. Dis., 1915, xvi, 385.

## SPECIAL MEDIA FOR THE TYPHOID-PARATYPHOID GROUP

The importance of differentiating the typhoid and paratyphoid bacilli from closely similar organisms, which live normally in the intestinal tract of man, has led to the development of many special media for the members of this group. The value of these media lies chiefly in the presence of certain carbohydrates which are acted upon by the ordinary intestinal bacteria, but are not utilized by the typhoid and paratyphoid bacilli. Among the numerous media devised for this purpose the following have proved of enduring value:

**Wurtz's Lactose Litmus Agar.**—Prepare ordinary slightly alkaline agar from meat infusion or meat extract, add 2 to 3 per cent. lactose (20–30 grams per liter), and enough litmus tincture to give a pale blue color. The typhoid and paratyphoid bacillus produces blue colonies, ordinary intestinal bacteria red colonies.

**Hesse's Typhosus Medium:**

| | |
|---|---|
| Agar, | 5 grams |
| Peptone (Witte's), | 10 grams |
| Liebig's beef-extract, | 5 grams |
| Sodium chlorid, | 8.5 grams |
| Distilled water, | 1000 c.c. |

The typhoid bacillus colonies are larger than those of *Bacillus coli* and may be several centimeters in diameter. They are circular and show small opaque centers and delicate opalescent bodies.

**Hiss's Typhosus Medium:**

*A. For plates:*

| | |
|---|---|
| Agar, | 15 grams |
| Gelatin, | 15 grams |
| Liebig's meat extract, | 5 grams |
| Sodium chlorid, | 5 grams |
| Dextrose, | 10 grams |
| Distilled water, | 1000 c.c. |

Prepare in usual way, clear with white of two eggs, and tube in 8 c.c. quantities.

*B. For tubes:*

| | |
|---|---|
| Agar, | 5 grams |
| Gelatin, | 80 grams |
| Liebig's meat extract, | 5 grams |
| Sodium chlorid, | 5 grams |
| Dextrose, | 10 grams |
| Distilled water, | 1000 c.c. |

Prepare in usual way, adjust reaction to 1.5 acid by addition of N/1 HCl. Clear with white of egg, filter, and add 1 per cent. dextrose.

Hiss: J. Exper. Med., 1897, ii, 677.

**Conradi-Drigalski Medium.**—*Original Directions.*—(*a*) Make an infusion of 3 pounds of meat in 2 liters of water by soaking twelve hours. Strain and boil one hour. Add:

| | |
|---|---|
| Witte's peptone, | 20 grams |
| Nutrose, | 20 grams |
| Sodium chlorid, | 10 grams |

Boil one hour and filter.

Add 60 grams agar.

Boil three hours or autoclave one hour till agar is dissolved. Make weakly alkaline to litmus. Filter through paper and boil one-half hour.

(*b*) Boil 260 c.c. litmus solution ten minutes.

(Kubel and Thiemann's litmus.)

Add 30 grams c. p. lactose.

Boil fifteen minutes. Decant if sediment forms.

(*c*) Add hot agar to hot litmus.

Mix, adjust to weak alkaline reaction by litmus paper.

(*d*) Add 4 c.c. of hot sterile 10 per cent. solution of sodium bicarbonate and 20 c.c. fresh solution of crystal violet (C. P. Höchst), made by dissolving 0.1 gram in 100 c.c. sterile $H_2O$.

Tube and sterilize.

**Park and Williams' Modification of Conradi-Drigalski's Medium.**—Prepare agar of the following composition:

| | |
|---|---|
| Agar, | 20 grams |
| Beef extract (Liebig's), | 4 grams |
| Sodium chlorid, | 5 grams |
| Peptone, | 20 grams |
| Nutrose, | 10 grams |
| Normal NaOH, | 50 c.c. |
| Water, | 1000 c.c. |

Dissolve in autoclave, cool, and clear with eggs. Make moderately but distinctly alkaline to litmus.

Add to each liter of agar so made:

| | |
|---|---|
| Kubel and Thiemann litmus solution, | 130 c.c. |
| Crystal violet (1 : 1000 solution), | 10 c.c. |
| Lactose, | 15 grams |

Heat in the Arnold sterilizer ten minutes to ensure complete solution, tube, and sterilize in the Arnold.

For the dysentery bacillus omit the crystal violet.

**Endo's Medium.**—1. Prepare 1 liter meat infusion 3 per cent. agar, containing 10 grams peptone and 5 grams NaCl.

2. Neutralize and clear.

3. Add 10 c.c. of 10 per cent. sodium hydrate solution to make alkaline.

4. Add 10 grams lactose (C. P.).

5. Add 5 c.c. saturated alcoholic basic fuchsin solution filtered before using.

6. Add 25 c.c. of 10 per cent. sodium sulphite solution.

7. Tube in 15 c.c. amounts, sterilize, and keep in the dark.

*Bacillus typhosus* produces colorless colonies, *B. coli*, red colonies.

Endo: Centralbl. f. Bakteriol [etc.], 1904, xxxv, 109.

**Kendall's Modification of Endo's Medium.**—1. Prepare 15 per cent. plain sugar-free meat infusion agar, making it just alkaline to litmus. Put 100 c.c. in flasks and sterilize in the autoclave.

2. Prepare a 10 per cent. solution of basic fuchsin in 96 per cent. alcohol.

3. Prepare a 10 per cent. aqueous solution of C. P. anhydrous sodium sulphite (1 gram in 10 c.c. water).

4. Add 1 c.c. of the indicator to 10 c.c. of the sodium sulphite solution and heat in the Arnold sterilizer for twenty minutes. This robs the fuchsin of practically all color, leaving it pink.

5. Add 1 gram of lactose (C. P.) to each 100 c.c. agar and dissolve in the autoclave.

6. Add enough mixture of indicator and sodium sulphite to make the medium faint pink.

7. Pour into Petri plates and allow to harden.

When properly prepared the medium is colorless when viewed from below and faint pink when viewed from the edge.

**Robinson and Rettger's Modification of Endo's Medium.**—Prepare agar of the following composition:

| | |
|---|---|
| Agar, | 25 grams |
| Peptone (Fairchild's), | 10 grams |
| Meat extract, | 5 grams |

Dissolve, make neutral to litmus, autoclave thirty minutes at 15 pounds pressure. Filter through cotton and cheese-cloth. Add 10 c.c. of 10 per cent. sodium carbonate solution, heat for ten minutes, add 1 per cent. lactose, 10 c.c. of 10 per cent. anhydrous sodium bisulphite, and bring reaction to +0.1 phenolphthalein by hot titration. Now add 5 c.c. saturated alcoholic fuchsin, tube in 20 c.c. amounts and autoclave seven minutes at 10 pounds pressure.

Park and Williams find that the best results are obtained by adjusting the reaction of the agar after the addition of the sodium carbonate, bottling in 100 c.c. amounts and adding the lactose, fuchsin, and sodium bisulphite, just before use.

**Loeffler's Malachite Green Medium.**—1. Prepare a neutral one-half strength meat infusion bouillon (500 grams of meat to 2 liters of water).

2. Acidify with 7.5 c.c. normal hydrochloric acid to facilitate solution of agar.

3. Add 30 grams of agar and boil.
4. Neutralize with 7 c.c. normal KOH or NaOH until neutral to litmus.
5. Add 5 c.c. normal sodium carbonate solution to make alkaline and heat in the Arnold sterilizer several hours.
6. Add 100 c.c. of 10 per cent. nutrose solution.
7. Sterilize and store in 100 c.c. quantities.
8. Before use dissolve and to 100 c.c. add 2 to 2.9 c.c. of a 2 per cent. solution of malachite green (Höchst 120) made in sterilized water, but not boiled.
9. Pour 15 to 20 c.c. into Petri plates and inoculate on surface.

Loeffler: Deutsche med. Wchnschr., 1906, xxxii, i, 289.

**Leuchs' Modification of Loeffler's Malachite Green Agar.**—1. To each 100 c.c. of one-half strength bouillon made by Loeffler's formula add:

| | |
|---|---|
| Sodium chlorid, | 0.5 gram |
| Dextrin, | 1 gram |
| Agar, | 3 grams |

2. Neutralize to litmus.
3. Add 0.5 c.c. normal sodium carbonate.
4. Add 10 c.c. 10 per cent. nutrose solution.
5. Boil, filter, and sterilize.
6. Add 1.6 c.c. of a 1/10 per cent. solution of malachite green, prepared by dissolving 0.1 gram in 100 c.c. hot sterile water.

Leuchs uses purified malachite green in crystallized form. This is not the zinc double salt, but the oxalate and is marked malachite green, pure, crystalline, oxalate.

Leuchs: Deutsche med. Wchnschr., 1906, xxxii, 2, 1330.

**Krumwiede's Brilliant Green Agar.**—Prepare two solutions, A and B.

*Solution A:*

| | |
|---|---|
| Agar, | 10 grams |
| Water, | 1 liter |

Dissolve in autoclave.

*Solution B:*

| | |
|---|---|
| Meat extract, | 6 grams |
| Salt, | 10 grams |
| Peptone, | 20 grams |
| Water, | 1 liter |

Dissolve in the Arnold sterilizer.

Mix A and B. Add normal soda solution bringing reaction to neutral to Andrade's indicator. This may require about 8 c.c. soda solution per liter. Preserve in 100 c.c. quantities and before using add to each 100 c.c. amount:

| | |
|---|---|
| Andrade's indicator, | 1 c.c. |
| Sterile lactose (20 per cent. solution in distilled water), | 5 c.c. |
| Glucose, | 0.2 gram |

and the appropriate amount of 0.1 per cent. solution of brilliant green in distilled water. Mix well, make thick plates, about 15 c.c. agar, and use Petri plates with porous tops to keep agar dry.

To determine amount of brilliant green required for each 100 c.c. agar, pour trial plates with the following solutions of the dye, 1 : 500,000, 1 : 330,000, 1 : 250,000, and 1 : 200,000, produced by adding to each 100 c.c. agar 0.2, 0.3, 0.4, and 0.5 c.c. of 0.1 per cent. solution. The amount of dye to be employed for routine work depends upon the organism investigated.

For the typhoid bacillus a dye concentration of 1 : 500,000 or 1 : 330,000 is suitable; for the paratyphoids a concentration of 1 : 200,000.

**Conradi's Picric Acid Brilliant Green Medium:**

| | |
|---|---|
| Liebig's meat extract, | 20 grams |
| Aqueous solution of Witte's peptone (10 per cent.), | 100 c.c. |
| Agar, | 30 grams |
| Water, | 900 c.c. |

Dissolve and filter.

Bring reaction to 3 per cent. acid to phenolphthalein (*i. e.*, each 100 c.c. would require 3 c.c. normal sodium hydrate to make it neutral). Sterilize and keep in bulk. Before use add 10 c.c. of a 1 : 1000 aqueous solution of brilliant green and 10 c.c. of a 10 per cent. aqueous solution of picric acid to $1\frac{1}{2}$ liters of agar. Pour into large Petri plates and inoculate by smearing the surface with suspected material. The colonies of *Bacillus typhosus* are light green, those of the paratyphoids are yellowish-green.

**Elsner's Medium.**—Grate up 500 grams potato in a liter of water, allow to stand over night, strain, and combine with a liter of 15 per cent. peptone gelatin, not neutralized. Add normal sodium hydrate till the reaction is fully acid to litmus, boil, filter, and sterilize. Before use add 1 per cent. potassium iodid.

**Peabody and Pratt's Malachite Green Broth.**—To 100 c.c. beef infusion broth add 10 c.c. 1 per cent. solution of malachite green (Höchst 120) made with sterile water. Tube in ten to 15 c.c. quantities.

Used as an enriching fluid for material suspected of containing the typhoid bacillus. Most favorable reaction $\frac{1}{2}$ per cent. acid to phenolphthalein.

Peabody and Pratt: Centralbl. f. Bakteriol. [etc.], 1908, xlv, 550.

**Conradi's Bile Medium:**

| | |
|---|---|
| Ox bile, | 900 c.c. |
| Glycerin, | 100 c.c. |
| Peptone, | 20 grams |

Sterilize in 100 c.c. quantities by fractional sterilization.

Conradi: Deutsche med. Wchnschr., 1906, xxxii, i, 58.

**Russell's Double Sugar.**—Add to meat extract agar made neutral to litmus, 1 per cent. lactose (10 grams), 0.1 per cent. glucose (1 gram), and litmus to give good color, or 1 per cent. Andrade's indicator.

Tube generous quantities (15 c.c.), slant and inoculate along surface by stabbing. Andrade's indicator may be used instead of litmus and the reaction adjusted to *p*H 7.2. This medium is red when hot, but decolorizes on cooling.

Russell: J. Med. Research, 1912, xxv (n. s. xx), 217.
Krumwiede and Kohn: J. Med. Research, 1917–1918, xxxvii (n. s. xxxii), 225.

**Brilliant Green Eosin Agar of Teague and Clurman.**—Prepare meat infusion agar, make +1 (acid) to phenolphthalein and autoclave before using. Now add:

| | Per cent. |
|---|---|
| Eosin, | $\frac{3}{50}$ |
| Brilliant green, | $\frac{1}{300}$ |
| Saccharose, | 1 |
| Lactose, | 1 |

*Bacillus typhosus* colonies on this medium grow large and have a grayish-pink color.

*Bacillus coli* when it grows produces colonies with deep red centers.

Meyer and Stickel use peptic digest agar and set the reaction at *p*H 7 – 6.8.

Stock dye solutions are made up as follows:

| | |
|---|---|
| Eosin yellowish, | 3 per cent. aqueous |
| Brilliant green, | 1 per cent. solution in 50 per cent. alcohol |

Of this use $\frac{1}{8}$ per cent. solution in distilled water.

Teague and Clurman: J. Infect. Dis., 1916, xvii, 647.
Meyer and Stickel: J. Infect. Dis., 1918, xxiii, 48.

**Eosin Methylene-blue Agar of Holt-Harris and Teague.**—Make up stock agar as follows:

| | |
|---|---|
| Agar, | 15 grams |
| Peptone, | 10 grams |
| Salt, | 5 grams |
| Liebig's extract, | 5 grams |
| Water, | 1000 c.c. |

This agar is cleared with white of egg, the reaction brought to +0.8 and then sterilized in the Arnold on three successive days. To the melted agar 5 grams of saccharose and 5 grams of lactose are now added, the medium being then heated in the Arnold for ten minutes. To every 50 c.c. medium 1 c.c. of 2 per cent. yellowish eosin and 1 c.c. 0.5 per cent. methylene-blue are added.

Holt-Harris and Teague: J. Infect. Dis., 1916, xviii, 596.

**Levine's Modification of Eosin Methylene-blue Agar:**

| | |
|---|---|
| Distilled water, | 1000 c.c. |
| Peptone (Difco), | 10 grams |
| Dipotassium hydrogen phosphate, | 2 grams |
| Agar, | 15 grams |

Boil to dissolve ingredients. Make up loss by evaporation. Fill into flasks, 100 c.c. to each. Sterilize in autoclave fifteen minutes at 15 pounds pressure.

Before use add to each 100 c.c. of medium:

| | |
|---|---|
| Sterilized 20 per cent. lactose solution 1 gram, | 5 c.c. |
| Aqueous 2 per cent. eosin (yellowish) solution, | 2 c.c. |
| Aqueous 2 per cent. methylene-blue solution, | 2 c.c. |

No adjustment of reaction or filtration is necessary. The dyes should be weighed on an analytical balance and added in the order indicated.

Levine: J. Infect. Dis., 1918, xxiii, 43.

## EGG MEDIA

Eggs are used as an enriching substance for media by a number of bacteriologists, especially in the study of tubercle bacilli and the pathogenic anaërobes. The most satisfactory are the following:

**Dorset's Egg Medium.**—Clean eggs with water and 5 per cent. carbolic acid solution, allow to dry partially, pierce the ends and blow into sterile Erlenmeyer flasks. Add 10 per cent. water by volume to the weight of the eggs. Mix gently, filter through cheese-cloth, tube, and slant. Coagulate by heating to 70° C. two to two and one-quarter hours on two successive days. Incubate to determine sterility.

**Lubenau's Glycerin Egg Medium.**—Blow two eggs into a flask and add 200 c.c. of 5 per cent. glycerin broth neutral or slightly alkaline to litmus. Tube, slant, and sterilize by heating to 70° C. two to two and one-half hours on two consecutive days. If medium is too dry, add a drop or two of water to supply the necessary moisture to each tube after coagulation, and seal with plugs soaked with paraffin or cork the tubes.

**Petroff's Gentian-violet Egg Medium.**—Add 500 grams of veal or beef to 500 c.c. 15 per cent. glycerin, extract twenty-four hours, squeeze out in a press and filter to render bacteria-free. Add 1 c.c. of a 1 per cent. alcoholic solution of gentian-violet to each 100 c.c. of extract; mix with 2 parts of beaten whole egg, tube inspissate and sterilize by heating three-quarters hour at 80° to 85° C. on three consecutive days. Especially suitable for the isolation of the tubercle bacillus from sputum, etc.

Petroff's medium may also be made without the addition of the gentian-violet and so prepared is valuable for the cultivation of the tubercle bacillus.

Petroff: Johns Hop. Hosp. Bull., 1915, xxvi, 276.

**Krumwiede's Alkaline Egg Medium.**—Mix equal parts of egg and water then mix equal parts of this egg mixture and 12 per cent. sodium carbonate (crystalline), and steam in the Arnold sterilizer twenty minutes. While hot add 30 to 70 parts of 3 per cent. agar (without meat extract), pour into plates and dry twenty minutes. To obtain larger colonies add meat extract or 0.5 per cent. glucose or saccharose.

Valuable for the typhoid bacillus.

Goldberger makes a less alkaline egg mixture with a 6.5 per cent. solution of anhydrous sodium carbonate, then mixes 1 part of this egg mixture with 5 parts of glucose extract agar.

## MEDIA FOR DIPHTHERIA TOXIN PRODUCTION

**Banzhaf's Medium.**—Add 1 pound of minced lean veal free from fibrous tissue to a liter of water. Soak over night and then heat to 45° to 55° C. for an hour. Bring to a boil, strain, and add 1.5 per cent. Witte's peptone (15 grams) and 0.5 per cent. sodium chlorid (5 grams). Boil one-half hour. Adjust reaction to 1.2 per cent. acid to phenolphthalein. Place about 800 c.c. in a 2 liter Erlenmeyer flask, add 3 grams of calcium carbonate and sterilize in autoclave at 15 pounds pressure one-half hour.

Park and Williams: 1920, p. 130.

**Diphtheria Toxin Broth from Martin's Peptone.**—Add 1 pound of minced lean veal free from fibrous tissue to a liter of water, incubate eighteen to twenty-four hours at 35° C. Heat to 45° to 48° C. for an hour. Boil briskly one-half hour. Strain through cheese-cloth. Measure, filtrate, and warm it to 70° C. Add an equal amount of Martin's peptone just neutralized at 70° C. Heat to boiling. Titrate at room temperature to +0.5 phenolphthalein. Autoclave at 10 pounds pressure fifteen to twenty minutes to clear. Titrate again and adjust to +0.5 if necessary. Filter through cotton and paper into flasks, allowing 800 c.c. to each flask. Sterilize one-half hour at 15 pounds pressure.

**Robinson and Meader's Tissue Broth for Diphtheria Toxin.**—Finely ground lean veal is infused in twice its weight of water and is stored in the ice-box for eighteen to twenty-four hours. It is then filtered through a wire gauze and heated to 80° C. for fifteen minutes. The infusion is thoroughly cooled and any fat present removed from the surface. Two per cent.

peptone and 0.5 per cent. sodium chlorid are added. The medium is brought to the boiling point and held there for five minutes. It is then titrated with phenolphthalein to from neutral to 0.3 per cent. acid and again boiled five minutes. It is now filtered through wire gauze and the sediment allowed to settle. It is distributed in 125 c.c. amounts in 300 c.c. Florence flasks and sterilized fifteen minutes at 15 pounds pressure. To each flask of meat infusion a small bit of sterile liver from rabbit or guinea-pig is added. The broth is used immediately as tissue which has been allowed to stand any time loses its power of enhancing toxin production.

Robinson and Meader: J. Infect. Dis., 1920, xxvii, 106.

### MEDIA FOR TETANUS TOXIN PRODUCTION

**Martin's Peptone.**—Add 1 pound minced lean veal free from fibrous tissue to a liter of water. Soak in ice-box eighteen to twenty-four hours. Heat to 45° to 48° C. for one hour. Boil briskly one-half hour. Strain through cheese-cloth. Measure filtrate, and warm to 70° C. Add an equal amount of Martin's peptone which has just been neutralized at 70° C. Heat to boiling. Titrate at room temperature to +1 phenolphthalein. Autoclave at 15 pounds pressure and clear. Measure and add 1 per cent. powdered glucose. Boil two to three minutes. Titrate again and set reaction as before to +1. Filter through cotton and paper directly into 2-liter Erlenmeyer flasks, leaving only sufficient space in flasks for expansion of broth during sterilization in the Arnold. Sterilize one and one-half hours on first day and one hour on second day.

**Park and Williams' Tetanus Toxin Broth.**—Add 1 pound of lean veal to 1 liter water, soak over night in ice-chest, heat to 45° C. one hour and then boil one-half hour. Strain, add 1 per cent. Witte's peptone, 0.5 per cent. salt and 1 per cent. glucose. Boil till ingredients are melted and adjust reaction to +1 per cent. Sterilize in 2-liter Erlenmeyer flasks in the Arnold, leaving only sufficient space in flasks for expansion during heating. Sterilize in the Arnold one and one-half hours on first day and one hour on second.

### MEDIA FOR THE INFLUENZA BACILLUS

**Chocolate Agar.**—Add a few drops of blood, preferably human or rabbit to ordinary melted agar and heat to 95° C. The final dilution of the blood should be about 1 to 30.

**Avery's Oleate Agar.**—Prepare the following materials:

1. Meat infusion agar +0.3 to +0.5 to phenolphthalein ($p$H 7.3 to 7.5).
2. Neutral sodium oleate, 2 per cent. solution in distilled water.
3. Washed rabbit red blood-corpuscles.

Melt the agar and cool to 45° C. Add 5 c.c. of the sodium oleate solution and 1 c.c. of the rabbit blood suspension to 94 c.c. agar. Pour into Petri plates and allow to cool.

Avery: J. Am. M. Ass., 1918, lxxi, 2050.

### MEDIA FOR THE PERTUSSIS BACILLUS

**Bordet and Gengou's Pertussis Medium.**—Add 500 grams sliced potato and 40 c.c. glycerin to 100 c.c. water. Heat in the autoclave one-half hour at 15 pounds pressure. Add to 500 c.c. of this material 1500 c.c. salt solu-

tion (0.6 per cent.) and 60 grams of agar. Autoclave to dissolve, filter, and tube. Just before use add an equal or less quantity of defibrinated blood.

## SPECIAL MEDIA FOR THE CHOLERA VIBRIO

**Hesse's Cholera Medium.**—Add 0.4 to 0.9 grams crystalline sodium carbonate to a liter of ordinary agar.

Hesse: Ztschr. f. Hyg., 1893, xv, 183.

**Dieudonne's Alkaline Blood Agar.**—Mix equal parts of defibrinated beef blood-serum and normal sodium or potassium hydrate solution; steam in the Arnold sterilizer half an hour. Add 3 parts of this to 7 parts of 3 per cent. agar neutral to litmus and pour into Petri plates, 15 c.c. to a 10 cm. dish. Let them harden uncovered, except by paper, place strips of filter paper between dishes and cover to absorb the ammonia, and incubate twelve to fifteen hours. Nothing will grow on the medium when first made and the plates are good for ten to fourteen days.

**Pilon's Modification of Dieudonne's Medium.**—Substitute 12 per cent. sodium carbonate for the sodium hydrate. The plates can be used after drying thirty minutes.

**Teague and Travis' Cholera Medium.**—Soak 2 pounds chopped beef in 2 liters distilled water in the ice-box over night. Squeeze out the fluid, heat in the Arnold, filter through filter paper, make neutral to litmus with sodium hydrate and heat again. Ferment with *Bacillus coli* to make sugar-free. Add 1 per cent. peptone, 0.5 per cent. NaCl and (1–2 per cent.) agar. Clear (with egg) and filter, adjust reaction to −0.5 phenolphthalein. Add 0.25 per cent. nutrose. To 50 c.c. of this nutrose agar add 1 per cent. saccharose, 1 c.c. 3 per cent. eosin solution from stock aqueous 3 per cent. bluish eosin solution kept in dark, and 2 c.c. 1 per cent. Bismarck brown solution made up in water containing 1 per cent. lactose. Distribute the dyes thoroughly by shaking and dry twenty minutes in the incubator upside down. Inoculate by smearing the surface. Cholera colonies are large with red centers, *Bacillus coli* colonies uniformly pink.

Teague and Travis: J. Infect. Dis., 1916, xviii, 601.

## SPECIAL MEDIA FOR THE CULTIVATION OF ANAËROBES

While obligate anaërobes grow at times on the usual media under anaërobic conditions, the experience of the past few years has demonstrated that the most important species require special methods for their cultivation. Many new media have been introduced for this group only a few of which have proved to offer special advantages. The following can be recommended.

**Cultures Containing Tissue.**—Many anaërobes grow well in the depths of solid or liquid media if bits of fresh sterile tissue are added to the medium, even if complete anaërobic conditions are not established. Freshly excised rabbit's kidney, spleen, or testicle, human heart muscle, bits of fibrin, the buffy coat of blood clot, and several other tissues may be used. The action of the tissues in favoring the growth of anaërobes is usually attributed to its reducing power. Inert foreign bodies like pieces of iron nails also favor the growth of anaërobes, so other principles than reduction may be involved.

**von Hibler's Brain Medium.**—Boil sheep or beef brain in equal amounts of water. Decant and press the brain through a potato grinder. Now add to the decanted water 2 per cent. peptone and 0.1 per cent. dextrose.

Heat and adjust reaction to slightly alkaline to litmus. Tube in large quantities, filling the tubes about one half. Sterilize in the Arnold for forty minutes on five consecutive days.

von Hibler: Untersuchungen über die pathogenen Anaëroben. Jena, 1908.

**Robertson's Cooked Beef Heart Medium.**—Take 8 ounces of bullock's heart, minced very finely and then ground in a mortar; add 8 ounces of tap water and heat slowly, so as to cook the meat thoroughly: add normal sodium hydrate until the mixture is alkaline to litmus. Divide into tubes and autoclave.

Robertson: J. Path. and Bacteriol. 1915–1916, xx, 327.

**Zinsser's Modification of Robertson's Cooked Meat.**—Place a few pieces of chopped meat in the bottom of a tube; add enough infusion broth with reaction *p*H 7.8 so that about 3 c.c. clear broth lie on top of the meat. Sterilize in the autoclave. Optimum reaction for the majority of anaërobic bacilli *p*H 7.4.

Kahn adjusts the reaction to *p*H 7.2.

Zinsser: A Textbook of Bacteriology. 1922, p. 168.
Kahn: J. Infect. Dis., 1922, xliii, 155.

**Holman's Meat Medium.**—Mix ground beef with equal quantities of water. Heat slowly to boiling. Neutralize and make slightly alkaline to phenolphthalein. Half fill tubes and place in autoclave one-half hour.

**Cooked Meat Fat-free Medium:**

*A*

Stir up ½ pound lean chopped beef in 250 c.c. of water. Neutralize the acids from the meat with sodium carbonate. Heat the mixture in an Arnold sterilizer thirty minutes with occasional stirring. Set in a cold place for several hours, then remove the fatty scum.

*B*

Mix the whites of 3 eggs with 250 c.c. water, neutralize, coagulate by heating in the Arnold for thirty minutes, stirring occasionally.

Mix A and B and add 2.5 grams of powdered calcium carbonate. Tube to height of about 2 inches. Sterilize in the autoclave thirty minutes at 10 pounds.

**Coagulated White of Egg Broth.**—A few eggs are boiled till the albumen is completely coagulated. The white of the egg is then carefully washed in distilled water to free it from the yolk, cut into small pieces, about 2 cm. in dimensions, and distributed in test-tubes. Broth of any special composition is now added in 10 c.c. quantities to the test-tubes and sterilized in the autoclave.

According to Kahn, beef infusion broth, plus 2 per cent. casein digest fluid with a reaction of *p*H 7.2, makes a good medium for egg cubes.

Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.

**Alkaline Egg.**—Mix equal parts of egg and water and add 12 per cent. crystalline sodium carbonate. Steam in the Arnold for twenty minutes. While hot mix 30 parts with 70 parts 3 per cent. beef-extract agar with unadjusted reaction.

**Robertson's Alkaline Egg Fluid.**—This is a modification of Besredka's medium. Beat up the yolk of one egg and the whites of two in a beaker;

add 6 c.c. of normal sodium hydrate, and 500 c.c. tap water by degrees. Heat very slowly to 95° C. keeping the mixture at this temperature for about an hour or longer; filter through cotton wool and muslin. Divide into tubes and autoclave at 115° C. for twenty minutes. This egg fluid may be added to ordinary nutrient broth in the proportion of about 1 : 5. The egg fluid must be added to the broth when both the fluids are cool. The egg fluid may be added to agar at 50° C.

Robertson: J. Path. and Bacteriol., 1915–1916, xx, 327.

**Kahn's Modification of Robertson's Alkaline Egg Fluid.**—The medium is made according to Robertson's directions and the reaction adjusted to *p*H 7.2. Some of the albumin does not go into solution but remains at the bottom of the tube in the form of a white precipitate. This modified substance has the appearance of a milky emulsion while Robertson's medium is golden-yellow in color.

Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.

**Kahn's Cooked Codfish Medium.**—Fresh codfish steaks are freed from bone and skin, chopped into fine particles and further reduced in size by a short period of grinding in a mortar. To each pound of fish 800 c.c. tap-water are added and the whole allowed to boil gently for forty-five minutes. The reaction is adjusted to *p*H 7.2. Prior to testing, the fish muscle and broth should be thoroughly mixed so that one vertical inch of the tissue may be present in each tube. Sterilize in the autoclave.

Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.

**Kahn's Inspissated Ox Serum.**—The serum is prepared from Difco powder, the reaction adjusted to *p*H 7.2 and the tubes inspissated vertically. This medium is inoculated by stabbing and serves for the study of proteolysis by anaërobes.

Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.

## SPECIAL METHODS FOR THE SPIROCHETES

**Noguchi's Method for Treponema Pallidum.**—Serum water, consisting of 1 part of serum and 2 parts of water, is put in test-tubes 20 cm. high in 16 c.c. quantities. Sterilized at 100° C. for fifteen minutes on three successive days. A small piece of sterile tissue put in each tube. Layer covered with sterile paraffin oil. After inoculation, incubated under strict anaërobic conditions which are best produced by a combination of hydrogen, vacuum, and pyrogallic acid.

Various types of serum may be used, the best being human, sheep, and rabbit. For the sterile tissue rabbit's kidney, testicle, or heart muscle are suitable, but not the liver.

Noguchi: J. Exper. M., 1911, xiv, 99.

**Noguchi's Solid Medium for Treponema Pallidum:**

*Composition:*

| | |
|---|---|
| Agar (2 per cent.) slightly alkaline, | 2 parts |
| Ascitic or hydrocele fluid, | 1 part |

The agar is melted, cooled to 50° C., mixed with the ascitic or hydrocele fluid. Bits of sterile tissue placed in test-tubes measuring 2 by 20 cm., then

covered with the melted medium, 15 c.c. to each tube. The agar is solidified by cooling and inoculated by puncture down to the piece of tissue. Medium covered with paraffin oil and incubated anaërobically. The organisms develop in hazy zones along the stab canal.

Different sterile tissues may be used. Rabbit's kidney and testicle are preferable, but human placenta, sheep's testicle, and other sterile organs also suffice.

Noguchi: J. Exper. M., 1912, xv, 90.

**Noguchi's Method for Cultivating Treponema Pallidum in Fluid Media.**—In this method two test-tubes are employed, connected by a perforated rubber stopper. The lower tube contains a piece of sterile tissue and is filled with a mixture of ascitic fluid and bouillon, no air being left. The upper tube contains a bit of sterile tissue and about 15 c.c. of solid medium made up of 2 parts of slightly alkaline 2 per cent. agar and 1 part of ascitic fluid, covered with sterile paraffin oil.

The fluid in the lower tube is first inoculated, some spirochætes being left about the tissue. The upper tube containing the piece of tissue is then connected, the melted medium poured in and this tissue also inoculated. The medium is covered with paraffin oil.

The organisms grow first in the solid medium and then migrate into the fluid medium below.

Noguchi: J. Exper. M., 1912, xvi, 211.

**Zinsser, Hopkins, and Gilbert's Method for Treponema Pallidum.**—Fluid serum agar is poured into 200 c.c. flasks, the bottom being covered with a layer about 1 inch in depth. Bits of sterile tissue, are added. Inoculated while still fluid. After cooling the agar is covered with a mixture of salt solution and heated ascitic fluid or slightly acid broth and ascitic fluid, up to the neck of the flask. A few bits of sterile tissue are floated in the fluid which is then covered with a layer of paraffin oil. Flask not sealed. After incubation two to four weeks large numbers of treponemata are found in the fluid.

*Pallidum* also cultivated in serum-broth mixtures in flasks with bits of sterile tissue added from fresh rabbit's spleen, and from fresh organs of rats and cats. For certain strains of *pallidum* autoclaved rabbit's kidney, liver, spleen, brain, lung, heart, and skeletal muscles were employed in fluid media made up of slightly acid meat infusion broth and heated sheep serum.

Zinsser, Hopkins, and Gilbert: J. Exper. M., 1915, xxi, 213.

**Schereschewsky's Gelatinized Horse Serum for Treponema Pallidum.**—Clear, sterile horse serum placed in small test-tubes, about the size of centrifugal tubes, three-fourths filled and tightly corked. Tubes placed in water-bath at 58° to 60° C. and kept at this temperature till a clear coagulum is formed. This semicoagulated medium is now placed in the thermostat at 37° C. for three days while partial autolysis takes place. Inoculated in the depths.

Schereschewsky: Deutsche med. Wchnschr., 1909, xxxv, 835.

**Noguchi's Method for the Spironemata of Relapsing Fever, Sp. Obermeieri, Duttoni, Kochi, and Novyi.**—Pieces of sterile tissue, preferably rab-

bit's kidney, are placed in test-tubes measuring 2 by 20 cm. A few drops of citrated blood from an infected mouse or rat are added and over this 15 c.c. of sterile ascitic or hydrocele fluid poured. The medium may be covered with paraffin oil but this is not necessary. Incubated aërobically.

Noguchi: J. Exper. M., 1912, xvi, 199.

**Hata's Method for Spironema Recurrentis.**—The blood of a normal horse is drawn off and allowed to clot. The serum in 4 c.c. quantities is placed in test-tubes of 1.5 to 1.7 cm. diameter. To each tube 8 c.c. normal NaCl solution are added, the total column of fluid being 6.5 to 7 cm. high. The medium is now placed in a water-bath at 58° C. and the temperature raised gradually till it reaches 70° to 71° C. in about three hours. Held at 71° C. thirty minutes. The contents of the tubes are converted into a translucent milky semisolid material, which after cooling becomes a semicoagulated mass. Pieces of rabbit's kidney are immersed in this and pushed to the bottom. The medium is inoculated with infected blood near the piece of tissue.

Instead of the rabbit's kidney the buffy coat of horse serum clot, cut into pieces 1 cm. in diameter, may be used. This buffy coat is composed of white blood-corpuscles and blood-platelets. Two or three pieces are pushed down to the bottom of the medium with a sterile glass rod and then inoculated with the spirochetes.

Hata: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1914, lxii, 107.

**Kligler and Robertson's Method for Spironema Obermeieri.**—Horse or rabbit serum diluted with 1 to 2 parts normal

| | |
|---|---|
| NaCl or undiluted ascitic fluid, | 10 c.c. |
| Peptone broth (10 per cent.), or egg-albumin, | 1 c.c. |

Reaction adjusted to *p*H 7.2.

Distributed in 3 to 4 c.c. quantities in test-tubes 1 cm. in diameter. Covered with a layer of paraffin oil 1.5 cm. in depth.

Inoculated with the blood of infected animals which furnishes the fibrin necessary for initial cultures. For subcultures a drop of fresh rabbit blood is added before the oil is poured over the medium. Incubated aërobically at 28° to 30° C.

An agar medium may also be used, 0.05 to 1 per cent. agar being added to the above mixture.

The spirochetes grow best at a reaction of *p*H 7.2 to 7.4. the limits of growth being *p*H 6.8 and 8.2. Since animal fluids like blood-serum become progressively alkaline the reaction must be carefully adjusted for satisfactory results.

Kligler and Robertson: J. Exper. M., 1922, xxxv, 303.

**Mühlens' Method for Spirochæta Dentium and Bacillus Fusiformis.**—Test-tubes half filled with slightly alkaline or neutral agar and kept in a water-bath half an hour to drive out the oxygen. Test-tubes of horse serum also kept in the water-bath half an hour at 58° to 60° C. Both fluids transferred to water cooled down to 45° C. Two parts of agar mixed with 1 part of serum. Inoculated at 40° to 42° C. with long platinum needle. Cooled and incubated at 37° C. nine to twelve days.

Mühlens: Ztschr. f. Hyg., 1906, lv, 81.

**Hogue's Medium for Spirochæta Eurygyrata:**

| | |
|---|---|
| Sterile salt solution (0.85 per cent.), | 15 c.c. |
| Serum water, | 0.3 c.c. |

Reaction adjusted to *p*H 7.

Either pig serum diluted 1 to 4 or sheep serum diluted 1 to 3 may be used but the former is better. Tubes should be covered with paraffin oil. Growth aërobic only.

Hogue: J. Exper. M., 1922, xxxvi, 617.

**Ita and Matsuzaki's Media for Leptospira Icterohemorrhagiæ:**

*Blood agar:*

| | |
|---|---|
| Nutrient agar, | 1–2 parts |
| Human or guinea-pig blood, | 1 part |

Mixed at 50° C. Inoculated while fluid with organisms from heart's blood of guinea-pigs infected with the blood or urine from cases of Weil's disease, with an emulsion of the liver or kidney, or with a small piece of the organs. Tubes covered with a layer of paraffin oil 2 cm. deep. Incubated at 15° to 37° C. Cultures left undisturbed ten to fourteen days.

*Blood gelatin:*

| | |
|---|---|
| Gelatin, | 1 part |
| Human or guinea-pig blood, | 2–4 parts |

Mixed at 25° to 30° C. Inoculated while fluid, covered with paraffin oil 2 cm. deep and incubated at 15° to 30° C. Left undisturbed ten to fourteen days.

Better results obtained with blood gelatin than with blood agar.

Ito and Matsuzaki: J. Exper. M., 1916, xxiii, 557.

**Noguchi's Media for Leptospira Icterohemorrhagiæ:**

*A*

| | |
|---|---|
| Rabbit serum, | 1.5 parts |
| Ringer's solution, | 4.5 parts |
| Citrate rabbit blood plasma, | 1 part |

Covered with paraffin oil.

*B*

| | |
|---|---|
| Rabbit serum, | 1 part |
| Ringer's solution, | 4.5 parts |
| Agar 2 per cent., neutral or slightly alkaline, | 1 part |

Covered with paraffin oil.

*C*

Semisolid portion

| | |
|---|---|
| Rabbit serum, | 1.5 parts |
| Ringer's solution, | 4.5 parts |
| Agar (2 per cent.), | 1 part |

Fluid portion

| | |
|---|---|
| Rabbit serum, | 1.5 parts |
| Ringer's solution, | 4.5 parts |

The semisolid portion is put in long-necked flasks of medium capacity, 50 to 100 c.c. The fluid portion is then poured over the surface. After inoculation covered with paraffin oil.

*D*

Fluid medium

| | |
|---|---|
| Horse or sheep serum, | 1 part |
| Ringer or NaCl solution, | 3 parts |

The organisms grow in an aërobic zone 1 to 1.5 cm. below the surface, at temperatures from 10° to 37° C. Tissue not needed and even undesirable. Value of serum destroyed by heating to 100° C. for fifteen minutes, diminished by heating to 60° C. for fifteen minutes, not injured by passing through Berkefeld filters. The nutrient principle is probably associated with coagulable serum proteins. Best growth obtained when at least 10 per cent. serum is present.

Noguchi: J. Exper. M., 1917, xxv, 755. *Ibid.*, 1918, xxvii, 593.

**Noguchi's Original Medium for Leptospira Icteroides:**

*Fluid portion:*

| | |
|---|---|
| Serum from non-immune humans, | 1 part |
| Ringer's solution, | 3 parts |

*Semisolid portion:*

| | |
|---|---|
| Serum from non-immune humans, | 1 part |
| Ringer's solution, | 3 parts |
| Melted neutral agar, | 0.3 per cent. |

Reaction of medium should be *p*H 6 to 7.4.

Eight c.c. of the semisolid portion put in tall culture tubes with 0.5 to 1 c.c. citrated blood from the patient or infected animal. Medium cooked and solidified. Eight c.c. of the fluid portion poured over the solid portion and also inoculated with 0.5 to 1 c.c. citrated blood. Surface covered with paraffin oil. In the original cultures the plasma forms a loose cobwebby fibrin clot in the fluid portion. For subcultures 0.5 to 1 c.c. citrate or human plasma are added. Organisms aërobic and grow best at 37° C.

Noguchi: J. Exper. M., 1919, xxx, 13.

**Noguchi's Improved Media for Leptospira Icteroides:**

*For isolation:*

| | |
|---|---|
| Fresh rabbit serum, | 100 parts |
| Distilled water, | 800 parts |
| Agar (2 per cent.) *p*H 7.5, | 100 parts |

To this medium the patient's blood is added in small quantities. It is hemolyzed by the distilled water and furnishes the necessary hemoglobin.

*For subcultures:*

| | |
|---|---|
| NaCl (0.9 per cent.), | 800 parts |
| Fresh rabbit serum, | 100 parts |
| Agar (2 per cent.) *p*H 7.5, | 100 parts |
| Rabbit hemoglobin, | 10–20 parts |

The rabbit hemoglobin is made by taking one part of defibrinated blood in ten parts of distilled water.

Noguchi, *et al.*: Monogr. Rockefeller Inst. M. Research, No. 20, August 9, 1924.

## SYNTHETIC OR PROTEIN-FREE MEDIA

**Cohn's Solution:**

| | |
|---|---|
| Potassium phosphate, | 0.1 gram |
| Magnesium sulphate, crystals, | 0.1 gram |
| Tribasic potassium phosphate, | 0.01 gram |
| Ammonium tartrate, | 0.2 gram |
| Distilled water, | 1000 c.c. |

Cohn: Beiträge zur Biologie der Pflanzen. 1872.

**Maassen's Solution:**

| | |
|---|---|
| Malic acid, | 7 gr. |
| Asparagin, | 10 gr. |
| Magnesium sulphate, | 0.4 gr. |
| Acid sodium phosphate, | 2 gr. |
| Pure soda (crystalline), | 2.5 gr. |
| Calcium chlorid (dry), | 0.01 gr. |
| Water, | 1000 c.c. |
| Potassium hydrate, | ½ to 1 per cent. |

**Proskauer and Beck's Protein-free Medium for the Tubercle Bacillus:**

| | Per cent. |
|---|---|
| Commercial ammonium carbonate, | 0.35 |
| Monobasic potassium phosphate, | 0.15 |
| Magnesium sulphate, | 0.25 |
| Glycerin, | 1.5 |

Sterilize twenty to thirty minutes three times in streaming steam.

This medium contains the substances regarded as necessary for the tubercle bacillus and in their proper concentration.

Proskauer and Beck: Ztschr. f. Hyg., 1894, xviii, 128.

**Uschinsky's Protein-free Medium:**

| | |
|---|---|
| Asparagin, | 3.4 grams |
| Ammonium lactate, | 10 grams |
| Sodium chlorid, | 5 grams |
| Magnesium sulphate, | 0.2 gram |
| Calcium chlorid, | 0.1 gram |
| Potassium phosphate, | 1 gram |
| Distilled water, | 1000 c.c. |

Completely dissolve and add 40 c.c. glycerin.

Uschinsky: Centralbl. f. Bakteriol. [etc.], 1893, xiv, 316.

**Fraenkel's Modification of Uschinsky's Medium:**

| | |
|---|---|
| Asparagin, | 4 grams |
| Ammonium lactate, | 6 grams |
| Disodium hydrogen phosphate, | 2 grams |
| Sodium chlorid, | 5 grams |

**Sullivan's Synthetic Medium:**

| | Per cent. |
|---|---|
| Asparagin, | 1 |
| Magnesium sulphate, | 0.02 |
| Dipotassium hydrogen phosphate, | 0.1 |
| Ammonium lactate, | 0.5 |
| Sodium chlorid, | 0.5 |
| Potassium nitrate, | 0.2 |
| Glycerin, | 1 |
| Agar, | 3 |
| Distilled water, | 1000 c.c. |

Sullivan: J. M. Research, 1905–1906, xiv (n. s. ix), 109.

## MISCELLANEOUS MEDIA

**Winogradsky's Solution for Nitrifying Bacteria:**

| | |
|---|---|
| Ammonium sulphate, | 0.4 gram |
| Magnesium sulphate, | 0.05 gram |
| Potassium phosphate, | 0.1 gram |
| Calcium chlorid, | Trace |
| Sodium carbonate, | 0.6 to 0.9 grams |
| Water, | 100 c.c. |

**Sabouraud's Media for Monilia:**

No. 1

| | |
|---|---|
| Distilled water, | 1000 c.c. |
| Maltose (brute de Chanut), | 40 grams |
| Peptone (granulé de Chassaing), | 10 grams |
| Agar, | 18 grams |

No. 2

| | |
|---|---|
| Distilled water, | 1000 c.c. |
| Glucose (massé de Chanut), | 40 grams |
| Peptone (granulé de Chassaing), | 10 grams |
| Agar, | 18 grams |

In both cases dissolve in autoclave at 120° C., filter through "papier chardin" (Cagit, 36 Boulevard Saint Michel, Paris). Place in wide test-tubes or Erlenmeyer flasks, sterilize at 120° C.

**Park and Williams' Mallein Broth.**—Add 1 pound minced lean veal free from fascia to liter of water; soak over night at room temperature. Heat at 45° to 50° C. for one hour. Boil up strongly. Strain through cheese-cloth. Add 1 per cent. Witte's peptone and 0.5 per cent. sodium chlorid. Titrate at room temperature, adjusting reaction to +2.5 phenolphthalein. Autoclave fifteen minutes at 15 pounds pressure to clear. Filter through cotton and paper. Measure and add 5 per cent. glycerin. Put 250 c.c. in quart Blake bottles or liter flasks and sterilize in autoclave one-half hour at 15 pounds pressure.

**Enlows' Sugar-free Medium for Fermentation Reactions.**—Enlows has devised a medium which may be used as a base for fermentable substances. It has a *p*H value of 7.2 to 7.4 at which reaction the pathogenic and allied organisms grow best.

| | |
|---|---|
| Tap (or distilled) water, | 1800 c.c. |
| Peptone, | 10 grams |
| $Na_2HPO_4$, | 14.5 grams |
| $KH_2PO_4$, | 1.4 grams |
| Agar, | 2 grams |

If brom thymol blue is employed as an indicator it may be prepared by dissolving 0.1 gram in 3.2 c.c. of N/20 NaOH to which 3 c.c. distilled water is added. About 3 drops should be added to each 500 c.c. of medium. The fermentable substances, sugars, alcohols, or glucosids may be added in quantities to give a 0.5 per cent. solution.

Enlows: Pub. Health Rep., 1923, xxxviii, 2129.

**Ellermann's Medium for Bacillus Fusiformis:**

| | |
|---|---|
| Agar, | 2 parts |
| Fluid horse serum, | 1 part |

Ellermann: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1904, xxxvii, Orig., 729.

## Chapter VI

# THE DESTRUCTION OF BACTERIA

### GENERAL CONSIDERATIONS

Bacteria are destroyed by diverse agencies which may have a *general* action, killing all types of organisms, or a *specific* action, killing only certain kinds. All these agencies must act upon bacteria by chemical or physical processes. On one side we have relatively simple chemical substances or physical procedures, on the other side very complicated cell structures. Chemically the cells consist of many simple and highly complex materials, partly in the form of fluid and semifluid colloids, partly as crystalloids, partly mixed. The death of the cell is chemically a lasting cessation of the anabolic and catabolic changes taking place in it normally, with an eventual decomposition of the fixed substances following the combined activity of autolytic ferments. It is generally believed that a cell poison can act only when it can pass through its capsule or membrane. It must be remembered, however, that a poison may spread about an organism, stop its assimilation and thus kill it.

The injury suffered by organisms can be of varying intensity and manifest itself in numerous ways. In its simplest form it is a temporary or inherited change in life processes, while reproduction, anabolic and catabolic activities go on as usual. Deeper changes may influence several or all the living functions, lengthen the period of growth and finally stop it completely. Under the influence of the injurious agencies the organisms cannot multiply, when removed from these agencies they multiply freely. Or the organisms can be injured more profoundly so that they are unable to multiply when favorable conditions are restored. We thus distinguish between *antiseptics* which restrain development and *disinfectants* which kill organisms. Among the disinfectants we differentiate between those which destroy vegetative bacteria and those which destroy spores. In general antiseptic value depends upon the intensity of the injurious action, *i. e.*, upon the concentration of the solution with chemical substances, while disinfectant or sterilizing value depends upon the time of exposure to the injurious agency.

Various methods have been devised to estimate antiseptic and disinfectant action. The antiseptic value of an agent may be determined by adding varying dilutions to cultures of the organism, finding the lowest quantity which will inhibit growth and noting the time of exposure. In general the vegetation of spores is easier to inhibit than the multiplication of vegetative forms. It is always necessary to utilize cultures containing the same number of bacteria, since a small number of organisms is naturally more easily influenced than a large number.

The temperature at which the experiments are carried out is especially important. Thus the efficiency of chemical disinfectants is increased the higher the temperature, while the antiseptic action is less marked at 37° C. than at 22° C. One must also take into account changes in the media, such as the acids or alkalies which may develop in it.

The sterilizing or killing power of a disinfectant is more difficult to determine than the antiseptic power. The bacteria must be freed from all traces of culture media, exposed to the substance to be tested for a definite period, freed from this test substance, and then restored to favorable conditions for multiplication to see if development is possible.

Disinfectants are usually tested for their power to destroy such vegetative organisms as the typhoid bacillus or the cholera vibrio, and such spores as those of *Bacillus anthracis*. Anthrax spores were originally used by Koch[1] because the identity of the organism could always be verified by animal inoculation. It has been pointed out by von Esmarck[2] and abundantly confirmed that different types of anthrax spores may show great differences in resistance to both steam and chemical agencies. Weil[3] has employed the spores of the red potato bacillus (*Bacillus mesentericus ruber*), developing a strain by systematic heating in which the spores show a constant resistance, about eight minutes to streaming steam. Hoffmann[4] used the spores of a saprophytic bacillus from garden earth. Vegetative bacteria in old cultures are more sensitive than those in young cultures and young cultures should always be employed (Ficker[5]). Different individuals in the same spore material show the greatest differences in susceptibility (Krönig and Paul[6]) and similar differences also exist in individual vegetative forms as in cultures of *Staphylococcus aureus* (Samter[7]). It is thus impossible to express any results absolutely. According to Paul and Krönig, Chick and Martin[8] the proper method is to work out the entire cycle of destruction, noting the time of expousre, the number of organisms killed, the number left over, etc. The destruction of the organisms takes place more and more slowly the farther the process goes on. With spores the number lessens in geometrical ratio with the increase of their resistance. These authors believe that the reaction conforms to the chemical law of mass action. Reichenbach[9] has shown that the survival of certain spores is to be traced to individual resistance. The same survival takes place when the resistance of spores to heat is determined, and here the law of mass action cannot be evoked. Furthermore, the destruction of spores in different species takes place according to quite different laws.

In testing the resistance of anthrax spores they are usually dried on silk threads according to Koch's original suggestion. This method is valuable for other species, especially those not destroyed by drying. The disinfectant must be washed off in water and the organisms then planted on culture media to determine the number of survivors. In many instances the organisms are injured so that they do not develop and yet are not killed. This condition is very difficult to establish practically. For bacteria that do not withstand drying an aqueous emulsion of spores may be used, but it is difficult to make emulsions uniform. Paul and Krönig advise drying the organisms in thin layers on plates. They can be exposed to the disinfectant, easily washed off, and the number of survivors determined.

The thermal death-point may also be determined by the use of sealed glass bulbs containing the organisms to be tested. These are known as Sternberg bulbs. They can be completely immersed in the heated water. After exposure to the requisite temperature the contents of the bulbs may be transferred to sterile tubes and incubated, cultures from the material may be made to determine the presence of viable organisms, or the surviving organisms estimated by colony counts. For material containing spores which require for their destruction temperatures above that of boiling water, the tubes may be immersed in oil which boils at temperatures above 100° C. or the tubes may be heated in the autoclave. In the determination of the

thermal death-point of organisms in the autoclave the time of exposure, the pressure and temperature and the length of time required to reach them should always be given in reports.

With chemical disinfectants equimolecular solutions should always be employed, that is, solutions which contain the same number of molecules in equal volumes or those in which the substances are dissolved according to their molecular weight. The concentration is thus given in the number of liters in which a gram-molecule of the substance is contained (a mol). For instance a 0.11 per cent. solution of sublimate is one in which 271.5 grams of sublimate are dissolved in 256 liters, the number 271.5 corresponding to the molecular weight of $HgCl_2$. The disinfectant must be washed off, neutralized in an acid or an alkali, or converted into some substances harmless to the bacteria.

The majority of bacteriologists advise that the tested material be planted on agar and the number of colonies counted on the third day. Colonies which develop later than this may come from injured organisms. To determine the death of all the organisms it may be necessary to grow the material in fluid cultures so that those few which do not form colonies on agar may be caught. At times the virulence of the organisms must be tested since rarely bacteria are present which can infect animals and yet not grow. This is the case with the plague bacillus.

In general different varieties or strains of the same species have the same susceptibility to chemical and physical agencies. Thus tubercle bacilli and certain of the pyogenic cocci are among the most resistant, the cholera vibrio and the gonococcus among the most susceptible. Certain species may be especially sensitive to disinfectants which have little influence on others. Thus the cholera vibrio is very sensitive to iodoform and both cholera and anthrax to malachite green.

The medium in which the chemical is dissolved is of great importance and results vary widely with water, alcohol, glycerin, oils, protein solutions, and body juices. Disinfectants dissolved in oil or in absolute alcohol have no action on bacteria. When oil is used the bacteria, which are largely water, are not moistened by the oil and so do not come in contact with the substance. Oil or fat may give up disinfectants to water and mixtures of the disinfectant with oil and water may have decided properties. This is to be considered in selecting the matrix of fats and oils for antiseptic salves, etc. In absolute alcohol the relations are very obscure. The lack of activity may be due to complete cessation of electrolytic dissociation. Dilute alcohol affects disinfectants differently. Many substances like phenol and formaldehyd become less active in alcoholic solution, while others like silver nitrate and sublimate have their activity increased by a moderate increase of alcoholic content and lessened by a higher concentration.

Combinations of physical and chemical disinfectants are often more active than either one alone. Von Esmarch[10] and Kokubo[11] have shown that the action of streaming steam is markedly heightened by the addition of small quantities of volatile chemical disinfectants to the water to be converted into steam. Thus the addition of 1 per cent. creosote, tricresol, acetic acid, or 0.1 per cent. formalin markedly raises the killing power of steam. Spores of the potato bacillus (*Bacillus mesentericus ruber*), which are usually killed in streaming steam at 100° C. in two hours, are killed in two minutes by steam to which 1 per cent. formaldehyd is added.

In all discussions concerning the destruction of bacteria by disinfection, especially by the chemical disinfectants, it must always be remembered that we can never reproduce conditions as they exist in the animal body.

The body fluids may neutralize the disinfectant and the bacteria in the body may be more resistant than they are in cultures, as Ficker has shown for the cholera vibrio.

## THE PHENOL COEFFICIENT

For accurate estimation of the disinfectant power of chemicals of the phenol series their destructive action on bacteria is compared with solutions of phenol in England and America. The ratio of the amount of the disinfectant needed to kill known organisms, compared with the amount of phenol required for the same organisms is known as the "phenol coefficient" as first described by Rideal and Walker.[12] In general a particular strain of the typhoid bacillus (Hopkins strain) is used as the test organism, cultivated in meat extract broth 1.5 per cent. acid to phenolphthalein. The reactions are usually carried out at 20° C. To 5 c.c. of the disinfectant dilutions 0.1 c.c. of a twenty-four-hour broth culture is added. At the same time a 5 per cent. solution of C. P. phenol is prepared and standardized by bromin titration. Of this, dilutions of 1 : 90, 1 : 100, 1 : 110 are made to serve as standards, and to each of these the same quantity of culture, 0.1 c.c., is added. These particular dilutions of phenol are selected because experience has shown that they suffice to kill standard quantities of the typhoid bacillus in brief periods of time, usually five to fifteen minutes.

Owing to the fact that different dilutions of phenol kill the test organisms at different periods the phenol coefficient of any particular disinfectant must

DETERMINATION OF THE CARBOLIC ACID COEFFICIENT OF A DISINFECTANT

(Anderson and McClintic)

Name ..... "A"
Temperature of medication ..... 20° C.
Culture used B. Typhosus ..... Twenty-four-hour extract broth, filtered
Proportion of culture and disinfectant ..... 0.1 c.c. + 5 c.c.
Organic matter, none; kind, none; amount, none.
Subculture media ..... Standard extract broth
Reaction ..... + 1.5
Quantity in each tube ..... 10 c.c.

| Sample. | Dilution. | Time culture exposed to action of disinfectant for minutes. | | | | | | Phenol coefficient. |
|---|---|---|---|---|---|---|---|---|
| | | 2½ | 5 | 7½ | 10 | 12½ | 15 | |
| Phenol | 1 : 80 | − | − | − | | | | |
| | 1 : 90 | + | − | − | − | | | 80)375 |
| | 1 : 100 | + | + | + | − | − | − | 4.69 |
| | 1 : 110 | + | + | + | + | + | − | 110)650 |
| | | | | | | | | 5.91 |
| | | | | | | | | 2)10.60 |
| Disinfectant "A" | 1 : 350 | − | − | − | | | | |
| | 1 : 375 | − | − | − | | | | 5.30 = |
| | 1 : 400 | + | − | − | − | | | coefficient |
| | 1 : 425 | + | + | − | − | − | − | |
| | 1 : 450 | + | + | − | − | − | − | |
| | 1 : 500 | + | + | − | − | − | − | |
| | 1 : 550 | + | + | + | − | − | − | |
| | 1 : 600 | + | + | + | + | − | − | |
| | 1 : 650 | + | + | + | + | + | − | |
| | 1 : 700 | + | + | + | + | + | + | |
| | 1 : 750 | + | + | + | + | + | + | |

naturally be estimated with these different dilutions as the standard. Unfortunately for the accuracy of the test, quite different results may be obtained with the same disinfectant depending on the time of exposure and the strength of the phenol. To render the test more accurate Anderson and McClintic[13] have modified it somewhat, using regularly two time limits, two and one-half and fifteen minutes, and comparing the strength of the disinfectant to be tested with the amount of phenol which kills the organism in these periods of time. The average of the two results obtained is known as the Hygienic Laboratory standard and is now employed almost universally in America in the standardization of disinfectants of the phenolic type. The table on page 151 illustrates the method advocated by the Hygienic Laboratory.

## CAUSE OF DISINFECTION

The destructive processes which lead to the death of the bacterial cells differ with different agencies. In some instances, as in the destruction of bacteria from drying or from immersing them in water, the osmotic changes set up are sufficient to kill sensitive organisms. In other instances the self-decomposition of the living bacteria is so increased that assimilation can no longer keep up with it. In this way we explain the bactericidal action of moderate heat (40°–60° C.) over long periods of time. Or the destruction may be due to oxidation of the living protoplasm so that it is completely burned up, as with chlorin, ozone, or light. Still other agencies operate by dissolving the bacterial substance as in the case of antiformin. In general the destruction of the bacteria is due to a coagulation of the living protoplasm, as with heat, metallic salts, phenol, and its derivatives. Weyland[14] believes that all energetic disinfectants which destroy spores are protein coagulants. With phenol an increase of the salt (NaCl) content results in an increase of the coagulation power for protein and an increase in disinfectant action. The bacterial cells also differ in their susceptibility to heat coagulation and this depends on their water content. Vegetative cells contain 80 per cent. water and are easy to coagulate and destroy. Spores consist of very concentrated protoplasm low in water and are thus hard to coagulate and destroy. Protein containing 6 per cent. water coagulates at 175° C., water-free protein at 170° C. Dry heat at temperatures of 140° to 170° C. brings out the same chemical changes in spores as are seen in the scorching of wool and cotton. Moist heat, especially saturated steam, gives up water to bacteria which are very hygroscopic when dried. The bacteria swell and their contents are coagulated.

We divide disinfectants generally into physical and chemical although both physical and chemical changes take place in cell destruction. Under the physical agencies the most important are light, drying, heat, and electricity. Under the chemical we include substances which act upon either the cell membrane or the cell contents by virtue of their chemical composition.

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## PHYSICAL DISINFECTANTS

**Light.**—The injurious action of light upon bacteria was first demonstrated by Downes and Blunt[1] in putrefying fluids, by Arloing[2] for anthrax bacilli, and by Koch[3] for *Bacillus typhosus*, *Bacillus coli*, and the cholera vibrio. Direct sunlight is more bactericidal than diffuse daylight, the direct sunlight killing bacteria in several hours (Kruse[4]), the diffuse daylight only after several days. The bactericidal action of light is greater in summer than in winter. Electric light is slower in its action than direct sunlight. The increase in temperature which results from exposure of bacteria to light is not the bactericidal agent, although it may assist in the destruction of micro-organisms. If all heat rays be absorbed by a thick layer of water or of alum, the bacteria in the depths are still destroyed by exposure to light (Dieudonné[5]). Buchner[6] has demonstrated a bactericidal action of light to a depth of 1.6 or even 3 meters. This action may play a rôle in the self-purification of water in streams. The activity of light lies in the violet end of the visible spectrum and in the ultraviolet rays. The ultrared and yellow rays have little or no action. The ultraviolet rays produced by passing sparks through high tension induction streams have strong bactericidal properties (Strebel[7]). Other bactericidal lights are iron light, electric light, light with iron electrodes, that from mercury steam flames and the light from sulphid of strontium. By immersing the bacteria in solutions of fluorescent dyes, like erythrosin, eosin, and fluorescin, the bactericidal action of the yellow rays is increased (Jodlbauer and von Tappeiner[8]). This action is not found in all sensitizing substances which act on photographic plates, only in fluorescent substances and in the presence of free oxygen. It may be explained by the fact that in sterile media, under the influence of the dyes, changes occur in the bacteria which make them sensitive to longer wave-lengths. Kruse[4] has found that the inhibition of bacteria by light is about equivalent to the action of a 0.25 per cent. solution of phenol. Various bactericidal chemical substances may be produced through the influence of light. Thus Duclaux[9] observed that formaldehyd develops from tartaric acid in lighted Raulin's fluid. Kruse has shown that these substances come from proteins only in the presence of free oxygen. In the presence of air hydrogen peroxid may arise in organic substrata (Richardson,[10] Dieudonné[5]), or organic peroxids (Freer and Novy[11]). Such substances are strongly bactericidal. The destruction of bacteria by light is not entirely explained by the production of bactericidal substances, since Ward[12] and Kruse[4] have shown that bacteria freed from media and dried are also destroyed by light, but with more difficulty than when rays of light are passed through the medium and the organisms introduced. The action of ultraviolet rays is not dependent upon the presence of free oxygen as has been shown by Bie[13] and Thiele and Wolf.[14]

Hertel[15] moreover has shown that the shorter the wave-length of the light to which bacteria are exposed the greater the bactericidal action and Browning and Russ[16] have found that with a tungsten arc line spectrum bacteria exposed to a wave-length of 296 mm. or less are killed. Thiele and Wolf further have observed that wave-lengths greater than 300 $\mu\mu$

are harmless to bacteria at 14° to 20° C. and lethal at 30° to 40° C. Recently Bayne-Jones and Van der Lingen[17] have been able to show that the upper wave-length limit of the bactericidal action of sunlight lies at 350 $\mu\mu$, the temperature at the time of exposure having no effect.

In hot climates light rays have some disinfectant power. According to von Esmarck[18] the destructive action is superficial and does not penetrate to the depths.

Roentgen rays have some bactericidal action on *Bacillus coli* and the cholera vibrio as has been shown by Rieder.[19] Long exposure, twenty to thirty minutes, is necessary and well grown cultures are more resistant than young cultures. It is doubtful whether bacteria in the tissues would be killed by *x*-rays.

Radium rays and radium emanations also have some action (Pfeiffer and Friedberger[20]). This action is found only in the group of radium rays easily absorbed, not in the group absorbed with difficulty.

For practical purposes ultraviolet rays have considerable value. They have been used successfully to destroy bacteria in water. They have little action in milk since they are largely absorbed by its ingredients.

Light has been frequently employed in the treatment of skin diseases like lupus (Finsen[21]). Here the beneficial effects may be due not to the destructive action of the rays upon the bacteria but to the tissue irritation which may cause a reaction against the organisms.

**Electricity.**—The electric current has no demonstrable bactericidal action. It is possible that substances like hydrogen peroxid may be produced in the menstruum in which the bacteria are exposed to the electric rays. The passage of the electric current through substances results in heat production and in general this is sufficient to account for any destruction of bacteria which may take place.

**Pressure.**—Increase of pressure of itself has no destructive action upon bacteria. Pressures of 500 to 600 atmospheres do not kill anthrax spores and *Staphylococcus aureus* is not killed by pressures up to 3000 atmospheres.

**Shaking** has little effect unless it be continued over long periods. After twenty-four hours certain species like *Staphylococcus aureus* are converted to a fine detritus and are killed in the process.

**Cold** of itself has little bactericidal action. Bacteria resist freezing and thawing and are usually not destroyed by the temperature of liquid air, 190° C.

**Desiccation.**—Many bacteria like the gonococcus and influenza bacillus are destroyed by drying. Koch[22] found that the cholera vibrio dried on coverslips was not alive after four hours but Berckholtz[23] observed them alive after twenty-four hours. The discrepancies in the results are to be explained by the different methods of drying which have been followed. The most exhaustive investigation of the effect of drying on bacteria has been made by Ficker.[24] The thickness of the layer of bacteria employed is of the greatest moment. The thinner the layer the more rapid the death of the organisms. Rapid drying in a desiccator causes a quicker death than drying in the atmosphere only when the organisms are spread out in thin layers. In thick layers the organisms survive in the desiccator longer than when dried in the air. Suspensions of bacteria in broth or milk dry with greater difficulty than in aqueous solutions. In general organisms die sooner at 37° C. than at lower temperatures. Drying occurs more quickly in moving air than when the air is free from currents. Old and weakened cultures are less resistant than young and active. Spores are far more resistant than vegetative forms.

The rapidity with which the moisture is withdrawn from the bacterial cell seems to be the factor determining the period of time bacteria resist desiccation.

**Heat.**—The most valuable agency for the destruction of bacteria is heat. Every increase of temperature over the minimum results in increased growth of micro-organisms and every increase over the maximum stops growth fully. In the latent life below the minimum the vitality is slumbering and the ability to live remains intact a long time. In temperatures above the maximum the inhibition is caused by catabolic or self-decomposition processes getting the upper hand so that assimilation can no longer keep up with them. An organism in a temperature above the maximum at which growth can occur, can not survive and the higher the temperature the more rapidly will death ensue.

In general, vegetative organisms are destroyed in a moist condition in ten to fifteen minutes at 55° to 60° C. Tubercle bacilli are more resistant and are destroyed in milk in fifteen minutes at 60° C. according to Hesse,[25] in twenty minutes according to Rosenau[26] and in fifteen to twenty minutes at 62° according to Theobald Smith.[27] Variations in the results of different observers are due to differences of technic. Thus concentrated emulsions of bacteria are more resistant than thin emulsions, especially if the latter are in salt solution. Older organisms are less resistant than young forms. Vegetative bacterial cells are killed by dry heat in one and one-half hours at 80° C. (Koch and Wolffhügel[28]). Anthrax spores are not killed by dry hot air at 100° to 120° C. in several hours, and are destroyed only at 140° C. after three hours. In boiling water or saturated aqueous steam at 100° C. they are destroyed in one to two minutes, at most in twelve minutes (Koch, Gaffky, and Loeffler[29]).

The differences between dry heat and moist heat are of great practical value. Expanded (gespanter) saturated steam (of more than 1 atmosphere and with a correspondingly high boiling-point) has more intensive action than unexpanded saturated steam at 100° C. Christen found that resistant spores of certain aërobic spore-bearing saprophytes (soil bacilli and potato bacilli) could survive over sixteen hours in unexpanded steam at 100° C., but were killed in expanded steam at 105° to 110° C. in two to four hours; at 115° C. in thirty to sixty minutes; at 120° C. in five to ten minutes; and at 140° C. in one minute. At the same time less disinfectant action is seen with saturated steam below 100° C. (by boiling under negative pressure), than with saturated steam at 100° C. Rubner[30] has shown that anthrax spores are killed at 100° C. in one minute, at 90° C. in twelve minutes, but not always at 85° C. in one hour.

With saturated steam the destructive action varies with the temperature, *i. e.*, the boiling-point. Unsaturated steam, *i. e.*, steam which in its volume contains a smaller number of water molecules than it should according to its temperature, is drier than saturated steam and its expansion force (Spannung) is less than it should be according to its temperature. Such unsaturated steam is easily produced by overheating saturated steam, as by throwing steam at 100° C. against strongly heated metal tubes. The disinfectant action of such superheated steam is much less than that of saturated steam of 100° C. (von Esmarck[31]). The activity is least at 120° to 130° C., gradually rises and only at 150° to 200° C. reaches its original. At such high temperatures dry air is itself disinfectant. Overheated steam at lower temperatures than 100° C. has less action (Rubner). Unsaturated steam is also mixed with air and in consequence the number of molecules of steam is less than it should be and the destructive power is correspondingly

less. Thus Rubner[32] has shown that certain spores which are killed in one to three minutes in saturated steam at 100° C. require three minutes for their destruction in steam mixed with 8.4 per cent. air, ten minutes with 20 per cent. air, and thirty minutes with 37 per cent. air. The disinfectant properties of hot air on the contrary are increased by the addition of steam. Thus Schumburg[33] has shown that hot air at 100° C. and 60 per cent. relative moisture kills all spore-free material in one hour, even tubercle bacilli in sputum.

The disinfectant power of moist heat is higher the nearer it reaches complete saturation and lower the less the saturation with steam and the more it approaches dry hot air. Coagulation of the protoplasm of the bacteria takes place at lower temperatures the greater the content in water. Dry bacterial bodies and almost dry spores can take hygroscopic water out of the surrounding medium the greater the medium is saturated, *i. e.*, the larger the number of steam molecules in the unit volume. The bactericidal action of dry heat at very high temperatures, 140° to 200° C., comes from combustion. Similarly wool and cotton at these temperatures undergo chemical decomposition with the liberation of $H_2S$ and the production of a brown color (Rubner).

Moist heat or steam is a better disinfectant than hot air because of its ability to penetrate porous objects like clothing. From this property comes its great value in disinfection. Koch and Wolffhügel have shown that the temperature was only 35° C. inside 19 woolen thicknesses after three hours heating by hot air at 130° to 140° C. By mechanical disturbance the air could be forced in but not enough for practical purposes. With steam the heat penetrates porous objects in two phases. First the air is driven out of the pores by the steam because of the difference in specific weight. Thus 1 liter of air at 20° C. weighs 1.206 grams, 1 liter of steam at 100° C. only 0.60 gram. When the steam is introduced from above the air is pushed out below (Gruber[34]). Secondly the steam condenses to water on the threads of the objects (clothing, etc.), and in the amount needed to warm the objects from their own temperature (usually about 20° C.) to the temperature of the steam. This concentration spreads centripetally in the form of a sharp zone of demarkation. The temperature necessary for disinfection is found only where the zone of demarkation is passed. At a point a few centimeters on the far side of the zone where there is no condensation, the temperature may be more than 40° C. lower.

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## CHEMICAL DISINFECTANTS

**True and Colloid Metals.**—The fact that metals as such are bactericidal was first shown by Miller[1] in preparations of gold used for filling teeth. Subsequently von Behring[2] demonstrated that if a piece of metal was put in a gelatin plate seeded with bacteria, certain organisms like the diphtheria bacillus, anthrax and *Bacillus pyocyaneus* ceased to develop in a zone about the metal, while other varieties like *Bacillus typhosus* and glanders grew only moderately. The bactericidal action remained after the metals were removed, suggesting that portions of them were dissolved. Bohtz[3] states that filtrates from metal powders are bactericidal. Credé[4] showed that thin silver plates placed on suppurating wounds and on agar plates of *Staphylococcus* disappeared, the silver being dissolved in the form of its lactic acid salt. Extremely small quantities of metals, not demonstrable by chemical tests, have a bactericidal action. Thus Ficker[5] has observed the oligodynamic action of copper, which can be recognized in a dilution of 1 : 50 million and can be demonstrated on the walls of glass vessels. Thiele and Wolf[6] state that the activity of a metal is increased when it is combined with electronegative metals (Pd. Pt. Au.) or with charcoal outside the medium, and is inhibited when it is combined with strong electropositive metals (Fe. Zn. Al. Mg.). Rankin[7] finds that the bactericidal action of metallic zinc in water is bound up with the presence of free oxygen. The action of true metals on bacterial cytoplasm has an analogy in chemistry since Schadee van der Does[8] has found that egg-albumin shaken with metallic silver is so charged in one-half to two minutes that it no longer clots by heat. This silver may be colloidal, which has antiseptic but slight bactericidal properties.

**Salts of the Heavy Metals.**—Of the salts of the heavy metals the mercury salts are the most valuable and have been most thoroughly studied. The bactericidal action of sublimate, $HgCl_2$, which was first recognized by Koch,[9] is not so high as originally supposed, but this substance is still the most valuable disinfectant known. It is antiseptic for anthrax spores in gelatin in a dilution of 1–1,000,000, in blood-serum in a dilution of 1–10,000 (von Behring[10]). A complete destruction of these spores was found by Krönig and Paul[11] in 1.69 per cent. solution in twelve to fourteen minutes, in 0.21 per cent. in eighty minutes, in 0.1 per cent. in two hours. In protein fluids the effect is slower (von Behring and Nocht), destruction occurring in 1 per cent. solution in eighty minutes, in 1 *promil.* solution not after

three hours, with certainty only after twenty-four hours. When $H_2SO_4$ is added in the proportion of 9 parts by weight to 1 part of sublimate, the spores are killed by 1 *promil.* sublimate solution after six hours.

Less favorable results than these were obtained by Ottolenghi,[12] who found that anthrax spores were killed in 2.7 per cent. sublimate solution in nine days while *Staphylococcus aureus* was alive up to nine hours in aqueous sublimate solution of this strength. In blood-serum the organisms were alive up to twenty-four hours. This resistance to the destructive action of sublimate is explained by the formation of a layer of mercury albuminate which protects the organism. To prevent this albuminate formation in protein solutions Laplace[13] and Panfil[14] recommend an addition of 5 per cent. tartaric acid or hydrochloric acid; von Behring[15] recommends the addition of salt.

Sublimate solutions must not come in contact with rubber since the combination of mercury and the sulphur in the rubber diminishes the disinfectant action and it may be entirely lost. Sublimate has an injurious action on surgical instruments and on the skin of the hands. Other mercury salts have been tried in its place like mercury oxycyanid ($HgO—Hg(CN)_2$) and sublamin (mercury ethyl diaminsulphate). The oxycyanid of mercury is more antiseptic than sublimate in blood-serum while sublamin, which is not so active as sublimate, does not clot protein in strong concentrations.

Other compounds of the heavy metals have occasionally been used. Thus the triamid of antimony thioglycollic acid and sodium antimony thioglycollate have been shown by Rowntree and Abel[16] to have a curative action in experimental trypanosomiasis. These disinfectants are now used with success in granuloma inguinale.

**Silver Salts.**—Next to salts of mercury silver salts are probably the most valuable disinfectants. Silver nitrate, $AgNO_3$, has an antiseptic value equal to that of sublimate. Its disinfectant value in aqueous solutions is less than that of sublimate but in blood-serum it is five times greater. Anthrax spores are killed in a 1–12,000 dilution of silver nitrate in blood-serum in seventy minutes. The addition of ammonia to silver nitrate solutions lowers its bactericidal action (Krönig and Paul[11]) since salts are formed in which the silver is present as part of complex ions, not as free ions. In salt solutions of silver nitrate the value of the agent is lessened by the formation of silver chlorid (AgCl). This is avoided in argentamin (ethylendiamin-silverphosphate). Argentamin is not precipitated in protein solutions containing chlorids. It is very active against certain vegetative bacteria like gonococci, but has no effect upon spores. Recently efforts have been made to combine silver nitrate with proteins in such a way as to give good disinfectants which produce little irritation and have greater penetrating properties. Some of these are argonin (argentamincasein), protargol (8.3 per cent. Ag), largin (11.1 per cent. Ag), albargin (15 per cent. Ag), and ichthargan (33 per cent. Ag).

**Alkalies.**—According to Paul and Krönig the disinfectant values of the alkali hydrates stand in direct proportion to their electrolytic dissociation, that is, the concentration of the hydroxyl ions (OH) in the solution. Thus potassium sodium and lithium hydrates are strong disinfectants, killing spores in eight to ten hours in simple normal solutions (concentration of 1 per liter) and killing staphylococci in 0.25 normal solutions in five to ten minutes. In ammonia, in which but slight dissociation takes place, there is little or no activity. This bears out the earlier observations of v. Lingelsheim. The activity of the alkali hydrates is much increased by heating. Von Behring[2] found that in ordinary washing soda with a soda content of

1.4 per cent., anthrax spores were killed at 85° C. in eight to ten minutes and at 75° C. in twenty minutes. Simon[17] states that 5 per cent. soda solution kills tubercle bacilli in sputum in five minutes, *Staphylococcus aureus* in fifteen minutes. Alkaline soaps have considerable disinfectant properties at ordinary temperatures, this being greatly increased at higher temperatures. Calcium hydroxid, $Ca(OH)_2$ (Aetzkalk), has powerful disinfectant properties because of its alkalinity. Neutral salts like calcium carbonate, which are produced when the hydroxid comes in contact with the carbon dioxid of the atmosphere, are inert. Milk of lime should always be used in freshly made solutions. For this purpose 600 c.c. of water may be added slowly to 1 kg. pure burnt lime (CaO). The calcium oxid decomposes slowly into a fine powdery mass of calcium hydrate. To this mass, which has a volume of about 2 liters, 8 liters of water are added so that one obtains a final product of 10 liters of 20 per cent. milk of lime. Such a fresh solution has a powerful disinfectant action and is especially valuable for the destruction of cholera vibrios and typhoid bacilli in stools, etc.

**Halogens.**—Of the halogens, chlorin is the most powerful disinfectant, killing anthrax spores in 0.2 per cent. solution in a few seconds (Geppert[18]), in 0.03 per cent. solution in two minutes, while in a 0.006 per cent. solution only a few spores are alive after five minutes. Bromin is almost as powerful as chlorin, killing anthrax spores in 0.06 per cent. solution in two minutes. Iodin is less active, not killing anthrax spores completely in 0.02 per cent. solution in five minutes. The addition of potassium iodid makes it less active. Nevertheless Lugol's solution (iodin in potassium iodid) is a valuable disinfectant in 0.01 to 0.05 per cent. solution. All halogens injure organic materials and break down rapidly in aqueous solutions. They are thus difficult to use in practice.

Certain combinations of the halogens are especially valuable. The addition of potassium permanganate to hydrochloric acid makes an effective disinfectant. Here the concentration of chlorin need not be so high since the combined chlorin is constantly released during the process of disinfection, being displaced by the new chlorin evolved. A combination of 1 per cent. potassium permanganate and 0.9 per cent. hydrochloric acid is more active than a 5 per cent. sublimate solution, killing anthrax spores in two minutes.

Chlorid of lime, consisting of a mixture of calcium chlorid ($CaCl_2$), calcium hydroxid ($CaOH_2$), and calcium hypochlorid ($Ca(ClO)_2$), in combination with acids and with atmospheric carbon dioxid produces hypochlorous acid which then splits off chlorin. In solution it kills vegetative organisms in concentrations of 0.1 to 0.2 per cent. Its activity is greatly diminished in media containing protein or salt. Typhoid bacilli are killed in feces in 1 per cent. chlorid of lime in ten minutes. Neutral and non-irritating solutions of sodium hypochlorite have now been prepared by a number of methods. The most widely used of these is Dakin's solution in which the bleaching powder is treated with dry sodium carbonate or washing soda.[19] The solution left after the precipitation of the calcium carbonate is now neutralized with boric acid. The final product contains about 4 per cent. sodium hypochlorite and before use is diluted with water so that it has a strength of 0.45 to 0.5 per cent.

In order to obtain chlorin antiseptics which give up their chlorin slowly when they come in contact with protein materials and so exert their destructive action on bacteria over a longer period of time, Dakin[20] has made a number of hypochlorite derivates that are soluble in oil; these have been extensively used in the treatment of wounds. The most important are

chloramin-T and dichloramin-T, in which chlorin has replaced the hydrogen atoms in the amino groups.

Antiformin, a mixture of sodium hydrate and sodium hypochlorite, has powerful disinfectant properties by virtue of dissolving the bacterial bodies.

Iodin trichlorid ($ICl_3$) is an active bactericidal agent and as strong as free chlorin. Cholera vibrios are destroyed in solutions of 1 : 2000 in one minute, anthrax spores in 1 per cent. solution almost instantaneously. This action is not lessened in media containing protein and salt. *Bacillus typhosus* and the cholera vibrio are killed in feces in *promil.* solution in fifteen minutes.

**Acids.**—The disinfectant power of acids varies with their grade of electrolytic dissociation, *i. e.*, with the concentration of hydrogen-ions in their solutions. Hydrofluoric acid (HFl), nitric acid ($HNO_3$), and trichloracetic acid ($CCl_3COOH$), have a specific action greater than indicated by their dissociation value. With increasing dilutions this action gradually falls behind the activity of their hydrogen-ions (Paul and Krönig[11]). The strong acids, perchloric acid ($HClO_4$), hydrobromic acid (HBr), hydrochloric acid (HCl), oxalic acid ($COOH_2$), and sulphuric acid ($H_2SO_4$), are strongly sporicidal, killing spores in five to eight hours by ordinary normal acid solutions. Sulphuric acid is the weakest, conforming to its lowest grade of dissociation. The acids of medium strength (phosphoric, formic, and acetic) disinfect according to their weaker dissociation grade, not killing spores in thirty hours.

For practical purposes raw sulphuric acid and hydrochloric acid are of value because of their cheapness. They lose in activity by combination with the objects to be disinfected and possibly by breaking down; hence they must be used in excess. Sulphurous acid is more active than sulphuric acid or phenol, but loses in strength from its volatility.

Organic acids have considerable disinfectant power. Citric acid in the concentration employed for lemonade (6 parts *promil.* citric acid + 50 *promil.* saccharose) kills cholera vibrios in fifteen to thirty minutes, typhoid bacilli in twenty-two to thirty-four hours.

**Oxidizing agents** disinfect according to their electrochemical relations. Chlorin is an exception to this rule and has a special bactericidal property, due not only to its power of oxidation but to its action as a halogen. Potassium permanganate in aqueous solution has a high disinfectant strength, killing anthrax spores in 4 per cent. solution in fifteen minutes, in 2 per cent. solution in forty minutes. Hydrogen peroxid is quite disinfectant, killing anthrax spores in 3 per cent. solution within sixty minutes, but it is readily decomposed.

**Neutral Salts.**—The neutral salts of the halogens have in general little ability to destroy bacteria. The chief discussions have arisen in regard to the amounts of salt (NaCl) and borax ($Na_2B_4O_7$) required to prevent the development of micro-organisms. Under thick layers of salt the cholera vibrios and spore-free anthrax bacilli die within twenty-four hours, while pus cocci, typhoid bacilli, tubercle and anthrax spores remain alive weeks and months. Complete inhibition of development was found by Lewandowsky[21] only in concentrations over 25 per cent. Borax has little activity being antiseptic only in $\frac{1}{2}$ to 2 per cent. solutions (Rolly[22]). In 5 per cent. solutions cholera vibrios are killed in seventeen hours.

**Organic Compounds of the Aliphatic Series.**—Carbon has no bactericidal action in itself but a number of carbon compounds have marked properties of this kind. Ordinary ethyl alcohol ($C_2H_5OH$) has an antiseptic action,

inhibiting many varieites of bacteria in 5 per cent. solution, and nearly all varieties in 10 per cent. solution. Spores are not destroyed at ordinary temperatures by absolute or dilute alcohol. Koch found that anthrax spores were alive after immersion several months in absolute alcohol. Boiling absolute alcohols, methyl, ethyl, and propyl, have almost no disinfectant power. Diluted with water they have uniform sporicidal properties within certain concentrations. When the alcohol concentration rises above a certain point, with methyl alcohol between 40 and 50 per cent., with ethyl alcohol between 80 and 90 per cent., with propyl alcohol between 90 and 100 per cent., the disinfectant efficiency falls to zero. Boiling propyl alcohol is most active between 11 and 40 per cent. (Saul[23]). Absolute alcohol has no action on dried vegetative bacteria, while 50 per cent. is the most powerful (Eppstein[24]). The same holds true for methyl alcohol. This difference is not evident with moist bacteria. Yersin[25] has shown that absolute alcohol will kill tubercle bacilli in cultures in five minutes and Schill and Fischer[26] have demonstrated that it will kill the same organism in sputum in twenty-four hours, when 4 parts of the alcohol are added to 1 part of the sputum. Pus cocci on silk threads are likewise destroyed in two minutes. The explanation of the difference in action between absolute and dilute alcohol lies partly in the precipitation of the protein in the outer layers of the bacterial cell by the concentrated alcohol, so that an impervious layer is formed which prevents the penetration of the alcohol into the interior of the cell.

Alcohol in a 50 per cent. solution has a disinfectant strength which lies between 1 *promil.* sublimate and 3 per cent. carbolic acid. It has an advantage over both in that it is less poisonous and has its activity increased by the addition of weak acids and alkalies, not diminished.

Acetone, chloroform, and ether have very slight bactericidal action.

Formaldehyd (HCOH), the aldehyd of formic acid (COOH), is now largely employed in practical disinfection, especially its 40 per cent. solution known as formalin. The antiseptic value of formalin is very high, even in protein solutions. Trillat[27] found it active in meat infusion in a dilution of 1 : 50,000 and completely *antiseptic* in a dilution of 1 : 12,000. This action varies with the species of bacteria, formalin inhibiting the growth of the cholera vibrio and glanders bacillus in a dilution of 1 : 20,000, *Staphylococcus aureus* in a dilution of 1 : 5000. The *disinfectant* power of formalin is much less. In a 2.5 per cent. solution in broth spore-free anthrax bacilli and cholera vibrios are killed in less than fifteen minutes, *Staphylococcus aureus* in fifty to sixty minutes. In a 5 per cent. solution it kills *Staphylococcus aureus* on silk threads in broth in thirty to thirty-five minutes, in a 2.5 per cent. solution in one minute. Heating increases its bactericidal power but even at 38° C. 10 per cent. formalin solution is not absolutely disinfectant. Anthrax spores are killed in 12.5 to 15 per cent. formalin at room temperature in about one and a half hours, at 35° C. in thirty minutes, at 52° C. in five minutes (Pottevin). Various commercial preparations of formaldehyd like lysoform have been widely employed. They all owe their disinfectant action to the contained formaldehyd.

Iodoform ($CHI_3$), long used by surgeons in dressing purulent surfaces, has little bactericidal power. Prompt destruction of bacteria is effected only with cholera vibrios. Tubercle bacilli are destroyed after two to three weeks contact with iodoform powder or after exposure to iodoform vapor for thirty to fifty days. Some antiseptic action is occasionally exhibited against *Staphylococcus aureus*, anthrax, mouse septicemia, and the diphtheria bacillus, but this action is inconstant. The favorable disinfectant or

antiseptic action seen in the treatment of wounds is explained by von Behring[28] in the theory that the reduction processes in putrefaction split off iodin from the iodoform, soluble iodin compounds being formed; these are bactericidal. Schmidt[29] finds that a decomposition of iodoform takes place in the presence of normal body fluids, possibly from the basic nucleus (Hexonkern) of the protein molecule. Heile[30] believes that the active disinfectant substance which arises from iodoform is a diiodacetylid, which is more powerful than sublimate. This may be the agent in iodoform poisoning. To get rid of the disagreeable smell of iodoform several compounds have been made, such as iodoformin, iodoformol, iodoformogen, etc. All owe their activity to the iodoform.

**Phenol and Cresol.**—Crude carbolic acid is a mixture of phenols and cresols. Phenol ($C_6H_5OH$) occupies an important place in disinfection. It is more expensive than sublimate and not so powerful, has an injurious action on the skin, causing a destruction of the epithelium, paræsthesia and gangrene. It is easily procured and is not injured by protein solutions, salts, acids, or alkalies. It has a stable chemical structure and its combinations with acids and alkalies are also disinfectant. Phenol and cresol ($C_6H_4(CH_3)OH$) precipitate protein slowly and a red color develops in impure carbolic acid which is of no moment in disinfection. The inhibition of bacteria with carbolic acid begins in a strength of 1 : 1250, and is complete in a dilution of 1 : 850 (Koch[9]). All vegetative bacteria are killed in 3 per cent. carbolic acid solution in eight seconds (Gärtner and Plagge[31]). Anthrax spores are not completely destroyed in 7 per cent. solution in thirty-eight days. Above this strength carbolic acid is not dissolved in water. After twenty-four hours the majority of the spores are killed. At 37° C. the effect is greater, all the spores being destroyed in 5 per cent. solution in three hours, in 4 per cent. solution in four hours, in 3 per cent. solution in twenty-four hours (Nocht[32]). The addition of hydrochloric acid, tartaric acid and neutral salts enhances the efficiency of carbolic acid. Alcohol lowers it and carbolic acid in absolute alcohol has no bactericidal properties.

There are three isomeric cresols, orthocresol, metacresol, and paracresol; their combination is called tricresol. Each of these cresols is more powerful than carbolic acid. Vegetative bacteria are killed by 1 per cent. orthocresol and paracresol in one minute, more rapidly by metacresol. The cresols are more poisonous than carbolic acid, particularly paracresol, and are only slightly soluble in water. Orthocresol dissolves in water in 2.5 per cent. solution, paracresol in 1.8 per cent., and metacresol in 0.5 per cent. Raw or crude carbolic acid, a thick brown cheap substance, is really raw cresol, the active ingredient being cresol. The pure cresols are obtained by mixing raw cresol with raw sulphuric acid or alkalies. After mixing with alkalies, active substances are obtained which do not injure the objects to be disinfected and are very valuable for surgical procedures. The most important are cresol soap, creolin which gives a milky emulsion with water, and lysol which gives a clear solution. Von Behring estimates that carbolic acid cresol and creolin in broth have a relative bactericidal action of 1, 3.5, and 10. In protein solutions the strength of creolin is reduced; in blood-serum it is fifty times lower than in broth and three to four times as low as carbolic acid. Anthrax spores are not killed in pure creolin in five weeks.

Lysol is more disinfectant, killing pyogenic cocci in 0.3 per cent. solution in thirty minutes, and anthrax spores slowly, in 0.5 per cent. solution in eight days. When lysol is heated to 55° C. the spores are destroyed in 10 per cent. solution in five minutes. In protein solutions the disinfectant action of lysol is lowered but not so much so as with creolin. A number of

products have been made with a chemical composition and a disinfectant action like that of lysol; sapocarbol, cresopol, bacillol, paralysol, etc.

Carbolsoap solutions may be made by combining 6 per cent. soft soap (green soap) with 5 per cent. raw carbolic acid and the active ingredient is some variety of cresol. It is cheap, and cleans and disinfects at the same time. Vegetative bacteria are killed in $1\frac{1}{2}$ per cent. solution in thirty minutes at ordinary temperatures. Anthrax spores are destroyed in a 5 per cent. solution in six hours at 50° C.

Certain other derivatives of phenol have a marked disinfectant action. Mercurophen, the oxymercury-orthonitro phenolate, according to Schamberg, Kolmer, and Raiziss,[33] kills *Staphylococcus aureus* in a dilution of 1 : 10,000,000. This preparation contains 55 per cent. of mercury.

**Dyes.**—Many of the organic dyes are strongly bactericidal as was first recognized by Koch. Of the triphenylmethane dyes, gentian-violet, malachite green, and brilliant green are the most toxic for bacteria. Malachite green (and cyanin) have an antiseptic action on anthrax spores in blood-serum in a dilution of 1 : 40,000, being more efficient than sublimate (von Behring[15]). Stilling in 1890 showed that a mixture of dyes known as pyoktanin, of which the active substance is supposed to be methyl violet, has an antiseptic action on various pathogens in a dilution of 1–10,000 (Stilling,[34] Jakowski[35]), being three times as powerful as the bichlorid of mercury. The flavine or acriflavine dyes are highly bactericidal, as first shown by Benda.[36] Crystal violet has an inhibitory action on cocci and certain bacteria, but no influence on *Bacillus coli* and *Bacillus typhosus* (v. Drigalski and Conradi[37]). The bacteriostatic action of gentian-violet has been thoroughly investigated by Churchman[38] and Churchman and Michael.[39] This dye has an especial effect on Gram-positive organisms like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus anthracis*, *Bacillus subtilis*, while it has little action on Gram-negative bacteria like *Bacillus coli* and *Bacillus typhosus*. Thus gentian-violet kills *Staphylococcus aureus* and *Staphylococcus albus* in a dilution of 1–100,000 in forty-eight hours.

Fraenkel[40] believes that the antiseptic value of the dyes depends on their general chemical composition, particularly on the aromatic nucleus, not on the groups of atoms which do the staining.

Several combinations of dyes and heavy metals have been made, some of which have marked disinfectant properties. The most important of these is "mercurochrome 220" or dibrom-oxymercury-fluorescein. This is prepared by substituting 1 atom of mercury in dibromfluorescein and contains 26 per cent. of mercury. According to Young, White, and Schwartz[41] this compound kills *Bacillus coli* and *Staphylococcus aureus* in a dilution of 1 : 1000 in one minute, *Bacillus coli* in a silution of 1 : 5000 in fifteen minutes, *Staphylococcus aureus* in a dilution of 1 : 10,000 in fifteen minutes. It has been used with success in local superficial infections, in cases of septicemia, in cystitis, and pyelitis.

**The Arsenicals.**—Various compounds containing arsenic have been used with great success in the treatment of protozoan and bacterial infections. Atoxyl or sodium paraminophenyl arsonate (Ehrlich and Bertheim[42]) has a favorable influence upon infections due to trypanosomes, upon experimental syphilis (Salmon, Uhlenhuth) and upon fowl spirillosis (Uhlenhuth). Salvarsan originally described by its discoverer Ehrlich as diaminodihydroxyarsenobenzol, contains trivalent arsenic and is a specific for syphilis in practically all its stages. This is now known as the arsphenamin base and is employed in practice in the form of its stable dihydrochlorid, which should possess an arsenic content of between 30 and 32 per cent. It is

necessary to add the proper amount of alkali to this product to convert it completely to disodiumdiaminodihydroxyarsenobenzol before injection. Sulpharsphenamin, first synthesized by Voegtlin and Johnson,[43] disodiumdiaminodihydroxyarsenobenzene, has also been used with success in experimental syphilis. It contains 19 to 24 per cent. of arsenic. Tryparsamid, the sodium salt of n-phenylglycinamido-p-arsonic acid, first synthesized by Jacobs and Heidelberger,[44] has been shown by Brown and Pearce[45], and Pearce[46] to have a favorable influence on experimental trypanosomiasis, and by Moore, Robinson, and Keidel[47] to be of value in the treatment of neurosyphilis and cardiovascular syphilis. It contains 25 per cent. of pentavalent arsenic.

**Miscellaneous Chemical Disinfectants.**—A number of chemical disinfectants have been prepared synthetically in the hope that they will have a specific action upon certain types of micro-organisms. Among them is *optochin*, ethylhydrocuprein, a derivative of hydroquinone, which has been shown by Morgenroth and Levy[48] to have a bactericidal action upon pneumococci acting in a dilution of 1 : 1,000,000 upon this organism. It also has a protective and curative action upon pneumococcus infections in small animals, guinea-pigs, and mice.

Chaulmoogra oil or ethyl esters of its fatty acids has been found by McDonald and Dean[49] to have a favorable action upon leprosy.

Ipecac and its alkaloid emetin have been used with favorable results upon protozoan infections, especially amebic dysentery and hookworm disease.

Bayer 205, a new remedy recently exploited in Germany for the treatment of sleeping sickness (trypanosomiasis), is believed by Fourneau, Tréfouel, and Vallée[50] to be the urate of aminobenzoyl-aminonaphthalenic acid.

Certain of the resorcinol derivatives have marked bactericidal action. The most powerful of these is hexylresorcinol first synthesized by Johnson and Hodge.[51] This compound has been shown by Leonard to have a phenol coefficient of 46. It has a powerful disinfectant action on *Staphylococcus aureus* which it destroys in a dilution of 1 : 100,000 in water and on *Bacillus coli* which it kills in a dilution of 1 : 12,000 in water in one hour. Hexylresorcinol is eliminated in an active state in the urine and has a specific curative effect on certain types of cystitis and pyelonephritis, especially those due to the pyogenic cocci (Leonard[52]).

## GASEOUS DISINFECTANTS

In the early days of bacteriology the theory was prevalent that contagion or infection was carried by the air and many gases were used for disinfection which were later shown to be merely deodorants. Certain gases like ammonia have an irritating effect upon the respiratory mucosa but no bactericidal action. Other gases like sulphur dioxid ($SO_2$), chlorin and bromin vapors, have decided disinfectant properties. Such substances have little effect on dried bacteria; when applied to moist surfaces they have an intense, destructive action on the objects to be disinfected.

By means of the Clayton apparatus, manufactured in England, large quantities of cool dry sulphur dioxid can be provided in rooms in a high concentration and an equable distribution. In this apparatus sulphur is burnt in a rich current of air, the resulting gas consisting of sulphur dioxid in a larger percentage than comes from the ordinary burning of sulphur, and possibly containing small quantities of complex oxidation products.

The Clayton gas is cooled and when still dry is driven into rooms from which the air is sucked out at the same time. The gas is thus forced into all the air spaces and has quite an activity in the depths. Gotschlich[53] points out that vegetative bacteria and insects like bedbugs, fleas, and lice are killed in thick mattresses and in clothing at a depth of 50 cm. in three hours in rooms 100 cubic meters in size when the gas content reaches 8 per cent. In larger rooms with 1000 cubic meters content similar results are obtained in twenty-four hours. The Clayton apparatus can be used for dwellings and especially for ships. Vegetative bacteria are nearly always killed in a concentration of 8 per cent. in a few hours but spores are not destroyed. Clothing must be covered with paper to prevent bleaching, metal objects oiled. Foods of all kinds, fruits, and meats are always ruined.

Sulphur dioxid is not as valuable a disinfectant agent as formaldehyd, which causes an extensive destruction of bacteria, has no harmful effect upon the objects to be disinfected, and is quite cheap. The gas does not penetrate to the depths reached by sulphur dioxid, but the penetration can be increased by warming to 50° to 60° C. In general, only surface disinfection is wanted. Where infectious material lies in the depths formaldehyd must be supplemented by substances like steam.

Formerly the active formaldehyd was obtained by allowing commercial formalin (40 per cent. formaldehyd) to evaporate spontaneously. In applying this method large quantities of formalin must be used for a long time in order to get the necessary concentration of formaldehyd. Two methods are available to furnish large quantities of formaldehyd. An aqueous solution can be evaporated rapidly, care being taken to avoid the formation of the polymerized formaldehyd (paraformaldehyd or paraform), or paraformaldehyd itself can be heated at 150° C. which suffices to convert it again to the active gaseous formaldehyd. Paraformaldehyd is prepared in pastilles which are easily heated to the desired temperature and can be employed for the disinfection of rooms. The bactericidal results are not especially valuable and it has now been shown that the air of rooms must be saturated with water vapor at the time the formaldehyd is used if complete destruction of bacteria is to be attained.

In Germany the Breslau method of formaldehyd disinfection is widely employed. In this method formaldehyd gas is produced by evaporating dilute aqueous formalin solution so that polymerization does not take place. Von Brunn,[54] believes that paraform is gradually formed in 20 per cent. solutions of formaldehyd, since these solutions give up less formaldehyd than aqueous solutions and so become more and more concentrated; with dilute solutions the reverse holds true. With about 7 to 8 per cent. formaldehyd solutions the relation between formaldehyd and water remains constant. This strength of formaldehyd is attained by the use of 1 part commercial formalin and 4 parts of water in the Breslau apparatus. This apparatus consists of a copper kettle with a narrow outlet tube in the cover, heated by a spirit lamp. It can be set up inside a room, or outside, the gas being introduced through the keyhole. The air in the room soon becomes saturated with moisture and the formaldehyd permeates the different objects. All known pathogenic bacteria including anthrax spores and tubercle bacilli are destroyed, provided they are not dried in layers too thick, within seven hours by $2\frac{1}{2}$ gm. formaldehyd per cubic meter, in three and a half hours by twice this quantity. To get rid of the formaldehyd gas after disinfection ammonia fumes are introduced which combine with the formaldehyd and form odorless hexamethylentetramin.

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## CHEMICAL DISINFECTION AS A CHEMICAL REACTION

In any consideration of the changes produced in bacteria by chemical substances the chief discussion has centered about such questions as to

whether the course of disinfection follows the laws of a chemical reaction, the type of physical changes which may be present and whether the cell membranes or the cell contents are attacked. Working on these questions Chick[1] came to the conclusion that the concentration of the disinfectant and its destructive power are reversely proportional. Later Madsen and Nymen[2] pointed out the similarity of disinfection to a monomolecular reaction, that is, a chemical reaction in which only one molecule undergoes a change, as in the splitting of cane-sugar by the hydrogen-ions of acids. With the number of the bacteria regarded as ordinates, the times of exposure as abscissas of an ordinate system, they obtained a curve in which the abscissas followed a convex line. The number of spores (destroyed or left over) dropped along a curve which had the abscissa axis as the asymptote, and theoretically could never become zero (Fig. 2).

The resemblance of the disinfection process to a monomolecular reaction was later confirmed by Chick, especially with the destruction of anthrax spores by sublimate solution. The work of Paul, Birstein, and Reuss[3] indicates that the action of a number of other disinfectants apparently follows the same law. The action of the disinfection increases with increases of temperature, just as the velocity of a chemical reaction increases with increased temperature. It is however usually two to three times greater. With such organisms as the paratyphoid bacillus the destruction by disinfectants did not follow the law of monomolecular reaction.

It has subsequently been pointed out by Reichenbach[4] that while disinfection in general follows the exponential law this may only be an accident. It has already been indicated that the survival of certain spores under the influence of disinfectants may be due to a higher individual resistance of these spores to the action of the injurious agencies. At the same time other factors may play a rôle, such as the combination of the disinfectant agent with the organism, the adhesion of the bacteria so that they are not reached by the substance, etc.

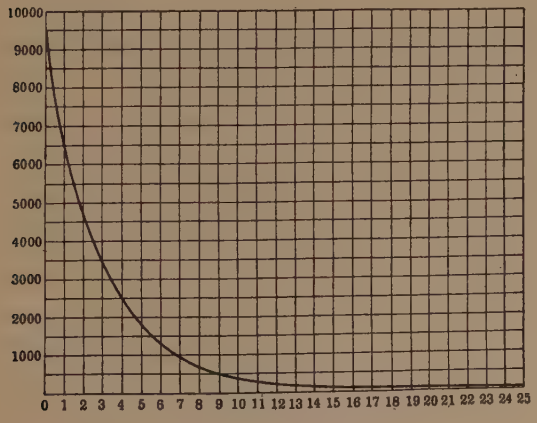


Fig. 2.—Madsen and Nymen curve.

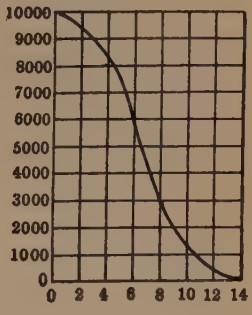


Fig. 3.—Eijkman's curve.

Finally Eijkman[5] has shown that the destruction of bacteria by heat follows the law of mass action, the curve obtained by determining the number of surviving bacteria being first concave, then convex (Fig. 3).

Ficker[6] believes that many processes of disinfection follow this law, which, it may be remembered, is followed by many biological phenomena.

Even if chemical disinfection be a process which follows the laws of chemical reactions, it is influenced by a variety of physical factors. The mere taking up of a substance by micro-organisms may bring on changes which lead to their death. Herzog and Betzel,[7] moreover, have shown that different disinfectants were taken up by yeast juice in different ways. Thus silver nitrate and chloroform were adsorbed, the adsorption exponent for chloroform being unusually large and quite out of the usual range. Formaldehyd was chemically bound in very small and constant quantities which were independent of the concentration. Phenol was simply dissolved. The chemicophysical relationship between cells and disinfectants thus differs widely with the different substances which have the capacity of destroying cells.

An emulsion of bacteria consists of a suspension of very numerous small bodies which have an enormous surface. When a substance is dissolved in the suspending fluid it may be *adsorbed* in which case the disinfectant is concentrated on the surface of the bacteria and the process follows the laws of diffusion (Henry's partition law). It is then taken up by the cells in relation to its solubility and lastly it combines with certain materials in the organism. In the interior of the organism *adsorption* may also play a rôle since here there are many colloid and crystalloid materials which are dissolved and present with each other in a half-fluid condition. Finally certain disinfectants seem to act like *lipoid solvents*. According to the lipoid theory of disinfection each cell is surrounded by a lipoid membrane and only those substances can get into the interior of the cell which are soluble in this membrane. Thus phenol is an active lipoid solvent and a powerful disinfectant. The salts of the heavy metals however have but slight solvent power for lipoids and yet have marked disinfectant properties.

Probably all processes play a rôle in disinfection, adsorption, solution in the bacterial membrane and cytoplasm, and chemical union.

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## PRACTICAL DISINFECTION

The disinfection of rooms and entire houses is best effected by the use of formaldehyd furnished by the Breslau apparatus or by sulphur dioxid from the Clayton apparatus. For single rooms formaldehyd candles suffice, provided a sufficient number are used. In many diseases the disinfection of dwellings is of no importance, since the causal organisms exist in the outer world, as in tetanus, or the infectious agents die rapidly under normal circumstances, as with gonorrhea and syphilis. Apparently there is little object in disinfection in influenza since the influenza bacillus has almost no capacity to survive outside of the body. Gotschlich[1] states that disinfection of dwellings is of value in three groups or types of diseases.

1. Diseases in which the causal organisms are carried by the air to every

part of the sick room are deposited on the surface of objects, but do not penetrate deeply. This group includes the exanthems of children, such as measles, scarlet fever, pertussis, influenza, diphtheria, and cerebrospinal meningitis. Here formaldehyd disinfection is of great practical value.

2. Diseases in which the organisms are carried by the air or by insects like fleas, lice, etc., and penetrate into the depths of porous materials. In this group we have tuberculosis, leprosy, smallpox, bubonic plague, typhus fever, recurrent fever, and certain septic infections. Here formaldehyd disinfection of the rooms should be carried out and mattresses and bedclothing sterilized by steam.

3. Diseases in which the organisms are not carried by the air but spread by the patient to bedclothes, body clothing, eating and drinking vessels, toilets, and floors. Here there is a deep contamination of the objects in the room and formaldehyd disinfection is of no value. In this group we have Asiatic cholera, typhoid fever, dysentery, and tuberculosis, etc. Here the contaminated objects should be thoroughly cleansed by washing with hot water and soap and treated with chemical disinfectants like creolin or sublimate solution. Bertarelli[2] proved that after spraying with 5 per cent. sublimate no sublimate is left in the air after one hour. A 2 per cent. soda solution heated to 60° C. is valuable for cooking utensils, floors, and wooden articles. Organisms like the tubercle bacillus are killed by this in five minutes. The walls should be washed with hot soap and water and then sprayed with sublimate. White-washed walls may be given a fresh coat of white-wash.

The chief discussion in regard to the disinfection of dwellings in which cases of contagious disease have occurred relates to the procedure known as terminal disinfection. Chapin[3] and other American authors believe that terminal disinfection can not be shown to have any definite effect upon the spread of many of the contagious diseases which pass rapidly from person to person. The practice of terminal disinfection leads to a false sense of security and to the neglect of routine cleansing by soap and water.

**Disinfection of the Secreta and Excreta.**—The dejecta of patients with typhoid fever, dysentery, and cholera should be treated with chlorid of lime, the urine with chemical substances like sublimate, carbolic acid, or lysol. The utensils which have contained feces and urine should be washed with caustic soda or with lysol. Disinfection of latrines may be accomplished with chlorid of lime, milk of lime being added in quantities equal to those of the fecal material. Ten grams of calcium chlorid suffice to disinfect 1 cubic meter of latrine contents. Fecal material may also be treated with acids (sulphuric acid, etc.) or mixed with such substances as peat bog which deodorize and disinfect at the same time.

**Surgical Disinfection.**—In the conduct of surgical operations all objects which can possibly come in contact with wounds should be rendered free of organisms, especial attention being paid to instruments and surgical dressings. The skin of the patient about the operative wound and that of the hands of the operator should be thoroughly cleansed and disinfected. Instruments may be boiled five minutes in 1 per cent. soda solution which suffices to kill the most resistant bacterial spores. Anthrax spores for instance are killed in two minutes by this procedure. Instruments may be sterilized by dry heat provided the time of exposure is not less than two hours. They may be sterilized by steam under pressure by exposure to the heat of the autoclave for fifteen to twenty minutes at 10 to 15 pounds pressure. Silk and metal ligatures should be sterilized by boiling. Silk ligatures may be impregnated with celloidin which gives a smooth surface and but few areas

where the bacteria may attach themselves. Catgut ligatures present the greatest problem in sterilization, since they come from animal tissues and may carry tetanus spores. Raw catgut carries tetanus spores in 1 per cent. of the samples (Bocchia[4]). Kleinertz[5] has reported over 30 cases of tetanus from the use of catgut. The spores apparently lie inside the threads of the catgut and their presence can only be determined by animal inoculation. Catgut is injured by boiling and the bacterial spores present are not reached by chemical disinfectants, since they are deeply embedded in fat. Various methods have been tried for the purpose of obtaining sterile catgut. It may be treated with Bergmann's solution, consisting of 80 parts of alcohol, 20 parts of water and 18 parts of sublimate. Absolute sterility is reached in two days. It may be boiled ten to thirty minutes in saturated aqueous ammonium sulphate solution (Elsberg[6]) or three hours in xylol. Krönig finds that the best results are obtained with catgut by boiling it in cumol which has a boiling-point of 168° to 178° C.

Surgical dressings and bandages are best sterilized by steam under pressure. An exposure for fifteen to twenty minutes to a pressure of 10 to 15 pounds usually suffices to render them free from bacteria.

The hands of the surgeon are especially difficult to render free from organisms. The bacteria live in the deeper layers of the skin, are impregnated with fat and are protected by the horny layer of the epidermis. They are almost universally present in the ducts of the sweat glands and about the hair follicles. They are frequent about the roots of the finger nails and under the nails. It is thus difficult if not impossible to completely disinfect the hands. A number of methods of hand disinfection have been devised. The hands should be washed in hot water and soap, scrubbed energetically with bristle brushes; the nails should be thoroughly cleaned. They may then be soaked in 1 : 1000 sublimate solution for at least five minutes. They may also be treated with 80 per cent. alcohol. The best results are obtained when the mechanical cleansing is followed by treatment with 80 per cent. alcohol for one minute, then by soaking in bichlorid for five minutes. They may also be immersed in a saturated solution of potassium permanganate, which is then removed by treatment with oxalic acid.

Owing to the great practical difficulty of rendering the hands bacteria-free, surgeons now employ rubber gloves almost universally. The gloves can be sterilized by boiling twenty-five to thirty minutes. They serve a double purpose, protecting the operative wound from any bacteria which may be present on the hands of the operator in such a location as to be beyond the reach of disinfectants, and protecting the operator from infectious material from the patient.

The skin of the patient should be mechanically cleansed by soap and water and then treated with some chemical disinfectant. The best results are obtained by saturating the surface with tincture of iodin, which is then washed off with alcohol. The skin of women and children is easier to disinfect than the skin of men. That of the covered parts of the body like the breast and abdomen is more easily disinfected than that of the exposed regions.

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## Chapter VII

## TAXONOMY

Modern classifications of both plants and animals rest upon the system of Linnæus,[1] published in 1773–1776, and it is significant that this author put all the minute microscopical forms in the single genus "chaos" despite the fact that he was a reluctant believer in the doctrine that living organisms might be the cause of putrefaction, fermentation, and disease.

The first observer to attempt a classification of microscopical organisms was apparently v. Gleichen[2] who investigated putrefying infusions of varied origin and for the first time employed stains or dyes to bring out morphological details. For this purpose he used solutions of indigo and carmin. The different types which v. Gleichen made out have now only an historical interest.

Soon after the Linnæan system was published Otto Friedrik Müller[3] in 1786 devised a classification according to the rules of Linnæus, and was probably the first to make use of a compound microscope for the study of bacteria. The entire lot of minute microscopical forms not included by Linnæus in his Worms, Müller put in the group Infusoria, separating them into organisms with outer organs and without outer organs and regarding them as belonging to the animal and not to the plant kingdom. The organisms without outer organs Müller again divided into the Crassinscula and the Membranacea, the crassinscula embracing the simplest types of cells and including what we now regard as the bacteria. Of these he made five genera, Monas, Proteus, Volvox, Enchelys, and Vibrio. Under Monas he described 10 and under Vibrio 31 different species. The separation of species was based by Müller on morphology, motility, origin or habitat, and certain biological peculiarities. From the chart which Müller published in 1786 it seems clear that he recognized the main morphological types of the bacteria and some of the names he introduced, such as Vibrio, Bacillus, and Spirillum, have survived in bacteriological nomenclatures, although with more exact or altered significance.

Following the observations of Müller no especial advances in the classification of bacteria were made for a period of about fifty years. During this time the science of bacteriology was developing slowly and gradually, helped by the interest in general science and facilitated by the improvements of the compound microscope which made accurate observations on morphology possible. It has already been indicated that the years 1837 and 1838 were notable for the wealth of contributions regarding the organisms found in fermentation and in certain parasitic diseases of the scalp. In 1838 Ehrenberg[4] attempted a more complete classification, employing the newer microscopes devised by Chevalier in Paris and by Pistor and Schick in Berlin. To bring out morphological details Ehrenberg made use of the method of "feeding" or staining organisms with powdered carmin or indigo introduced by v. Gleichen, a device which often revealed a complicated structure in the forms. He regarded even the simplest infusoria as animals rather than as plants and sought to prove this by his morphological studies. Since particles of the stains used by Ehrenberg collected in the interior of the organisms in hollow spaces, which he regarded as stomachs, he named

the entire group Polygastrica, but included in it a large number of forms in which he had not observed any such inclusion of stain particles. Ehrenberg made three families, Monadina, Cryptomonadina, and Vibrionia. It is difficult to say how many of the organisms now regarded as bacteria were included by Ehrenberg among the Monads, but one at least, described as *Monas prodigiosus*, is evidently so treated. All the members of the third group Vibrionia were so clearly described and illustrated as to leave no doubt of their bacterial nature. Ehrenberg regarded the Vibriones as Monads which by incomplete segmentation formed threads. He divided these Vibriones by their morphology and flexibility into four main genera with 14 species. The straight non-flexible forms Ehrenberg included in the genus Bacterium but the three species in this genus can not now be recognized. The slightly curved serpentine flexuous cells he put in the genus Vibrio of which he described six species, only one of these surviving as *Vibrio subtilis* (*Bacillus subtilis*). Of the organisms forming true spirals or screws Ehrenberg made 2 genera, placing the flexuous forms in the genus Spirochæta and the rigid forms in the genus Spirillum. The genus Spirochæta included only one species, *Spirochæta plicatilis*, which can still be regarded as a valid species, while in the genus Spirillum three species were described, *Spirillum tenue*, *Spirillum undula*, and *Spirillum volutans*. Of these, *Spirillum undula* was Müller's *Vibrio undula*. A fifth genus, Spirodiscus, was formed only tentatively, resting upon a single observation of a peculiar type of spiral found in water.

Ehrenberg's classification was based upon such definite observations and was withal so simple that it was generally accepted by his contemporaries. There can be little doubt that he saw and described the main morphological types of the rod-shaped bacteria as we recognize them today. This classification met with opposition later at the hands of the French investigator, Dujardin,[5] who adopted Ehrenberg's families, combining them in a single group of "animals without visible organs of locomotion, moving by virtue of their general contractility." This family Dujardin divided into three genera, Bacterium, Vibrio, and Spirillum. The straight rigid forms capable only of vibratory movement he called Bacterium, the straight or slightly curved forms endowed with a serpentine motility he called Vibrio while the corkscrew threads moving by rotation about the long axis he called Spirillum. He thus discarded entirely Ehrenberg's genus Spirochæta. All these genera Dujardin put in the Zoöphites, raising the question nevertheless whether these minute microscopic forms belonged to the animals or to the plants.

The doubt expressed by Dujardin as to the proper position of the bacteria in any classification of living things was later emphasized by Perty[6] who concluded that these minute microscopic forms belonged sometimes to the animal and sometimes to the plant kingdom, some being intermediate in position, part plant and part animal. The Vibriones could properly be linked up with the Algæ as plants and these Perty divided into Spirillina and Bacterina. The Spirillina included the Spirochæta and the Spirillum as defined by Ehrenberg and the Bacterina included the Vibrio and the Bacterium as defined by Dujardin.

The various attempts to classify the bacteria devised by Ehrenberg, Dujardin and Perty helped to clarify a confused situation but it remained for v. Nägeli[7] and Cohn[8] to carry out investigations of such a broad and fundamental character as to lay a firm foundation for modern toxonomy. In 1849 Nägeli investigated the single-celled Algæ with great care and emphasized the morphological similarity of the colorless microscopic forms with the pigmented Algæ. He later separated the pigmented from

the non-pigmented bacteria, putting the pigmented among the Algæ, the non-pigmented among the Fungi, chiefly on the basis of their physiological reactions. Thus the Algæ are able to manufacture the substances necessary for building up their protoplasm from the elements, carbon, oxygen, hydrogen, and nitrogen, which are present as carbon dioxid, ammonia and water in the materials surrounding them and in certain salts, to combine with or bind oxygen in the presence of sunlight and at the same time to manufacture green chlorophyl or some related pigment. The Fungi on the other hand are like animals and the majority of parasitic plants and are unable to take the substances necessary for their life processes from inorganic material, but must find higher organic combinations ready for their utilization. They can therefore flourish only on living or dead organic material, or in water which contains definite quantities of such substances. They do not excrete oxygen and do not turn green in the light. All the organisms which show absence of coloring-matter, live on organic material and fail to excrete oxygen, must be included among the Fungi. On the basis of these considerations Nägeli in 1857 put all the microscopic colorless forms, Bacterium, Vibrio, Spirillum, Sarcina, the so-called mother-of-vinegar (*Umbrina aceti*) and the yeast-like structures found in a disease of the silk worm (*Nosema bombycis*), in one common group which he named Schizomycetes. He left the question open as to whether the members of this group should be regarded as plants, animals, or combinations of plants and animals.

Despite the fact that Nägeli did not employ a proper basis for a true classification, as was later pointed out by Cohn, he did perform a service of inestimable value in bringing together the many different types of microscopical organisms now regarded as bacteria in a single group with certain characters common to all.

In the development of bacteriology following Nägeli's work the most significant advances were made in regard to the morphology and the cultivation of the bacteria. Thus the form Sarcina, discovered in 1842 by Goodsir[9] and included by Nägeli in the Schizomycetes, was observed by several investigators, including Virchow,[10] Heller,[11] Frierichs[12], and Welcker,[13] in various secretions of the animal body and recognized by its characteristic appearance. Its peculiar morphology was now shown by Suringar[14] to depend upon a regular method of segmentation in three directions of space. Potato, which had been introduced for the cultivation of Ehrenberg's *Monas prodigiosus* by Fresenius[15] and later employed by Hoffman[16] for the cultivation of both pigmented and non-pigmented strains, led in Schröter's[17] hands to a differentiation among the various types of organisms known as the Bacteria or the Bacteridia. Some of these forms produced red pigment, some blue, and some brownish-green. This may be regarded as the first definite separation of different species of micro-organisms on the basis of cultural or physiological characters.

Directly following the work of Schröter on the pigmented bacteria the investigations of Cohn[18] in the Botanical Institute of the University of Breslau had the greatest significance. Cohn had already concluded that the Vibriones belong to the plants, being closely related to the Algæ and properly included in the group of water Fungi (Mycophyceæ). In 1870 he described *Crenothrix polyspora*, for which he made a new genus Crenothrix to include organisms going through a more complicated development than that enjoyed by the simpler bacteria. Two years later Cohn[19] brought together in the group of *Bacteria* a large number of microscopical organisms the common characters of which were their freedom from chlorophyl, their spherical or cylindrical shape, their division by transverse fission, and their

arrangement as isolated individuals or as cell families. The colorless Oscillaria (Beggiotoa) he separated completely from the Bacteria. The cell protoplasm of the bacteria according to Cohn was flexuous, the cells possessed membranes which were not protein but cellulose or a closely related carbohydrate, were not destroyed by acids or alkalies and resisted putrefaction. These membranes were directly visible under the microscope, were capable of swelling, and were either rigid or flexuous but not so much so as to be truly serpentine. True branching did not occur and spores or resting stages were suspected, especially in some of the forms with swollen terminals. The Bacteria were divided by Cohn into four tribes for which he retained the older names, each of these tribes having one or more genera as follows:

Tribe I: **Sphærobacteria** (spherical bacteria).
Genus 1 *Micrococcus* char. emend.

Tribe II: **Microbacteria** (rod-shaped bacteria).
Genus 2 *Bacterium* char. emend.

Tribe III: **Desmobacteria** (thread-formed bacteria).
Genus 3 *Bacillus* nov. gen.
Genus 4 *Vibrio* char. emend.

Tribe IV: **Spirobacteria** (screw-shaped bacteria).
Genus 5 *Spirillum* Ehrenberg.
Genus 6 *Spirochæta* Ehrenberg.

The Sphærobacteria were small or oval forms, often forming chains as a result of transverse division. Division in two directions of space was not mentioned. These forms were divided by Cohn into three groups according to their physiological characters, the chromogenic, xymogenic, and pathogenic. The chromogenic organisms were separated into two groups, those with pigment soluble in water and diffusible through the medium in which the organisms grow (*Micrococcus cyaneus*, *Micrococcus violaceus*) and those with insoluble pigments which remained in the protoplasm or intracellular substance (*Micrococcus prodigiosus*). The Microbacteria were differentiated from the Sphærobacteria by their elongated or cylindrical morphology and from the Desmobacteria by their failure to form threads. They included the single genus Bacterium with two species, *Bacterium termo* and *Bacterium lineola*. The Desmobacteria growing in threads included two genera, Bacillus for the elongated forms and Vibrio for the bent or curved forms. Two species of Bacillus were described, *Bacillus subtilis* and *Bacillus ulna*, and two vibrios, *Vibrio rugula* and *Vibrio serpens*. The vibrios were separated from the next group, Spirillum, by their wavy motility. The Spirobacteria included the genus Spirochæta with flexible long closely-wound spirals and the genus Spirillum with stiff short and open spirals. In the genus Spirochæta he recognized one species, *Spirochæta plicatilis*, and in the genus Spirillum three species, *Spirillum tenue*, *Spirillum undula*, and *Spirillum volutans*. In this last species Cohn observed unstained flagella on both ends of the spiral.

The great difficulty in the establishment of a proper classification of the bacteria was well recognized by Cohn since such a small number of characters existed for this purpose. Even with the greatest magnification provided by the microscopes then available it was impossible to make out with sufficient clearness their form, the cell contents, or their mode of reproduction. This was especially true since no definite life cycle had been proved for the bacteria according to which the species were perpetuated by eggs or spores. Cohn clearly recognized the fact that until individual bacteria could be

isolated, cultivated, and observed under a great variety of conditions, no method was available to determine the developmental stages of the bacteria, their species and varieties.

As to the proper place of the bacteria in the world of living things Cohn could find no evidence of their relation to animals and had already put them definitely among the plants in the group Thallophytes among the Cryptogams.[20] Of the Thallophytes Cohn made seven different orders instead of the three previously established, namely, the Fungi, Lichens, and Algæ. The first of these orders was composed of the Schizosporeæ, including the Schizomyceteæ (Bacteriaceæ) and the Schizophyceæ. With the Fungi the Bacteria had in common only their lack of chlorophyl or an equivalent pigment and their inability to assimilate carbon dioxid. In morphological and developmental characters the Bacteria were not related to the Fungi, but to the blue-green Algæ from which they were separated only by their characteristic motility and their freedom from chlorophyl.

In this period other investigations were reported which came to have an important bearing upon classification. The most significant were those of Klebs[21] who found micrococci in pathological material from various sources and of Billroth[22] who observed what we now regard as streptococci in infected wounds. While both these observers failed to interpret their observations correctly, the group of Micrococcus first proposed by Hallier was given its proper importance by Klebs and a new group of chained cocci established by Billroth. About this time also the fundamental researches of Pasteur upon spontaneous generation and upon fermentation had opened the way to a broader comprehension of the rôle of the bacteria in various fields of biological activity.

Taking account of all these newer investigations of others and of the work going on in his own institute Cohn[23] developed in 1875 a complete scheme for the classification of the bacteria. He placed them among the lower Algæ as the group Schizophytæ with two main tribes, the Gloeogenæ and Nematogenæ.

While Cohn's elaborate classification has for us now chiefly an historical interest we must not underestimate the profound influence it had upon systematic bacteriology. For the first time the organisms grouped among the bacteria were placed side by side in an orderly manner on the basis of their morphological characteristics, the members of these morphological groups being separated from each other by their physiological reactions. This method of classifying the bacteria has persisted up to the present time and the groups which Cohn established are in the main accepted today as the proper divisions of the bacteria. In the years immediately following the publication of Cohn's system the most important discovery was made by Cohn[24] himself when he found spores in *Bacillus subtilis* and separated the genus Bacillus from the non-sporulating rods. As we have already seen this was followed by Koch's discovery of spores in *Bacillus anthracis* and by his development of those technical methods which made modern bacteriology possible.

From time to time during the next few years systems of classification were proposed by Zopf,[25] Schröter,[26] De Toni and Trevisan,[27] Hueppe[28] and a number of others. None of these new systems proved to possess any clear advantage over that of Cohn and none of them found any wide acceptance except that of Zopf. In 1884 and 1885 Zopf introduced a simple scheme of arranging the groups of bacteria chiefly on the basis of morphology. He made four primary groups, all being included among the Fission Fungi as follows:

*Fission Fungi*

| | |
|---|---|
| Coccaceæ | Streptococcus. |
| | Merismopedia. |
| | Sarcina. |
| | Micrococcus. |
| | Ascoccus. |
| Bacteriaceæ | Bacterium. |
| | Spirillum. |
| | Vibrio. |
| | Leukonostok. |
| | Bacillus. |
| | Clostridium. |
| Leptothriceæ | Crenothrix. |
| | Baggiotoa. |
| | Phragmidiothrix. |
| | Leptothrix. |
| Cladothriceæ | Cladothrix. |

Eisenberg,[29] a little later, arranged the bacteria in simple groups based upon cultural characters and pathogenic action, and provided investigators in bacteriology for the first time with a comprehensive scheme by means of which unknown micro-organisms could be identified. From this time cultural reactions and pathogenic properties have occupied an important place in toxonomy.

During this period many improvements in the technical methods for the study of the bacteria were introduced from time to time, especially in relation to the demonstration of the cilia or flagella. In 1890 Messea[30] proposed to use flagellation as a means of separating genera. He divided the bacteria into:

I. Gymnobacteria, without flagella.

II. Trichobacteria, with flagella.

1. Monotricha, with 1 polar flagellum at 1 end.
2. Lophotricha, with a bunch of flagella at 1 end.
3. Amphitricha, with flagella at both ends.
4. Peritricha, with flagella distributed about the entire body.

Migula[31] now employed flagellation for a classification of the bacteria upon a broad and comprehensive basis, devising a scheme which has found wide acceptance among bacteriologists. All the spherical forms were put in the Coccaceæ, the rare motile micrococci and sarcinas being separated from the non-motile forms as Planococcus and Planosarcina. The straight cylindrical organisms were put together in the group Bacteriaceæ and divided into the non-motile rods as Bacterium, the motile rods with peritrichic flagella as Bacillus, and the motile rods with terminal or polar flagella as Pseudomonas. For the spiral organisms Migula made four genera in the family Spirillaceæ, putting the non-motile spirals in the Spirosoma, the motile spiral forms with 1 to 3 polar flagella in the genus Microspira, the motile spiral forms with a bunch of polar flagella in the genus Spirillum. The twisted corkscrew spirals, moving possibly by an undulating membrane, were left in the genus Spirochæta. The more complicated organisms which pass through different stages of development, sometimes called the Higher Bacteria were included by Migula in a new group Chlamydobacteriaceæ with five genera, Streptothrix, Cladothrix, Crenothrix, Phragmidiothrix, and Thiothrix. The genus Leptothrix previously established by Kützing was discarded.

Despite the fact that Migula, in working out his system of classification,

employed old terms with a new significance not always strictly in accord with the original meaning of the term, as in his use of Bacillus and Bacterium, the classification he established has been largely followed by bacteriologists all over the world. This is partly due to the subsequent publication of his *System der Bakterien* in which he arranged in an orderly manner nearly 2000 species of micro-organisms described by hundreds of different observers. We give it therefore in full.

## BACTERIA

Phycochrome-free fission fungi dividing in one, two, or three directions of space. Many varieties have endospores. When motility is present it is due to flagella-like organs of locomotion or to an undulating membrane, the latter indicating a relation to the Phycochromaceæ.

I. Family **Coccaceæ:**

Cells truly spherical when free. Division in one, two, or three directions of space, each spherical cell dividing into halves, quarters, or octants which later develop into spheres. Endospores rare.

1. Genus *Streptococcus*. Cells divide in one direction of space forming bead-like chains when they remain in contact after division. No organs of locomotion.

2. Genus *Micrococcus*. Cells divide in two directions of space, forming grape-like clusters when they remain in contact after division. No organs of locomotion.

3. Genus *Sarcina*. Cells divide in three directions of space, forming ball-like packets when they remain in contact after division. No organs of locomotion.

4. Genus *Planococcus*. Cells divide in two directions of space, as in *Micrococcus*, but possess flagella.

5. Genus *Planosarcina*. Cells divide in three directions of space, as in *Sarcina*, but possess flagella.

II. Family **Bacteriaceæ:**

Cells long or short, cylinders straight, never screw-like. Division in one direction of space after lengthening of the rods.

1. Genus *Bacterium*. Cells without organs of locomotion, often with endospores.

2. Genus *Bacillus*. Cells with flagella arranged about the entire body. Often possess endospores.

3. Genus *Pseudomonas*. Cells with polar flagella. Endospores present in only a few species.

III. Family **Spirillaceæ:**

Cells spirally wound or forming parts of a spiral. Division in one direction of space after previous lengthening in the long axis.

1. Genus *Spirosoma*. Cells rigid, without flagella.

2. Genus *Microspira*. Cells rigid, with one, rarely two to three wavy polar flagella.

3. Genus *Spirillum*. Cells rigid, with polar tufts of 5 to 20 flagella, forming half circles or open spirals.

4. Genus *Spirochæta*. Cells flexuous, serpentine. Organs of locomotion unknown, possibly an undulating membrane.

IV. Family **Chlamydobacteriaceæ:**

Organisms going through varied stages of development, all possessing a firm membrane or sheath surrounding cells which are united in branched or unbranched threads.

1. Genus *Streptothrix.* Cells joined in simple unbranched threads. Division only in one direction of space. Multiplication by non-motile conidia.

2. Genus *Cladothrix.* Cells united in threads showing pseudodichotamous branching. Division only in one direction of space. Vegetative development by formation of complete branches. Multiplication by swarming bodies with polar flagella.

3. Genus *Crenothrix.* Cells combined in unbranched threads with division originally in only one direction of space. Cells divide later in three directions of space. The products of division become spherical and grow into developmental cells.

4. Genus *Phragmidiothrix.* Cells originally combined in unbranched threads dividing in three directions of space and thus forming a kind of rope of cells. Later the individual cells can grow through the very thin delicate sheath which surrounds them and undergo true branching.

5. Genus *Thiothrix.* Unbranched non-motile threads enclosed in delicate sheaths, with division of the cells in one direction of space. Cells contain sulphur granules.

V. Family **Beggiotaceæ:**

Cells united in threads without sheaths. Division in one direction of space. Motility by an undulating membrane as in the oscillaria.

Genus *Beggiotoa.* Cells with sulphur granules.

Soon after the publication of Migula's classification of the bacteria on the basis of motility and flagellation a similar attempt to use these characters was made by Fischer.[32] His classification however has not been widely followed.

The type of arrangement of the bacteria according to cultural reactions and pathogenic action, originally proposed by Eisenberg, was brought to perfection by Kruse[33] in 1896. Kruse divided the bacteria into a fairly large number of natural groups, organisms being brought together on the basis of physiological properties. While Kruse's system may be regarded as somewhat artificial, it proved to be of great help to the student and investigator in the identification of unknown organisms.

In 1896 Lehmann and Neumann[34] introduced a simple arrangement of the bacteria which restored the earlier significance of many of the terms employed and has served a useful purpose. They followed Kruse for the Coccaceæ and the Bacteriaceæ and Loeffler and Migula for the Spirillaceæ. The Coccaceæ Zopf, emended by Migula, were divided into three genera, Streptococcus of Billroth, Sarcina of Goodsir and Micrococcus of Cohn. They thus discarded the general Planococcus and Planosarcina devised by Migula for the motile spherical organisms. In the Bacteriaceæ Zopf emended by Migula, identical with the Bacillaceæ of Alfred Fischer, they made two genera, Bacterium Cohn emended by Hueppe for the non-spore-formers and Bacillus Cohn emended by Hueppe for the spore-formers. Flagellation was thus discarded as a generic character and spore-formation restored to its proper place in classification. For the spiral bacteria they adopted Migula's Spirillaceæ, making however only three genera, Vibrio Müller emended by Loeffler for the short slightly bent rigid comma-like organisms with 1 to 2 terminal flagella, Spirillum for the long spirally wound rigid organisms with a bunch of polar flagella, and Spirochæta of Ehrenberg for the flexible long spiral threads moving possibly by an undulating membrane. The great advance made by Lehmann and Neumann lay in the recognition that certain organisms like the tubercle bacillus and the diphtheria bacillus exhibit a complicated structure which is quite different from

that of the simpler bacteria and entitles them to separate treatment. On the basis of this consideration Lehmann and Neumann established the genus Corynebacterium for the diphtheria bacillus and organisms resembling it, the genus Mycobacterium for the tubercle bacillus and other acid-fast bacteria. These two genera were put in the family Actinomycetaceæ together with the genus Actinomyces as defined by Harz and emended by Gasperini. The higher Fission Fungi or Fission Algæ were divided by Lehmann and Neumann tentatively into five genera, Leptothrix Kützing, Beggiotoa Trevisan, Crenothrix Cohn, Cladothrix Cohn, and Thiothrix Winogradsky.

Finally in 1909 Jensen[35] proposed an entirely new method of classification in which physiological properties were made the basis for the division of bacteria into orders, families, genera, and species. This classification of Jensen's has not been thus far adopted.

In America the most important contributions to taxonomy are those of Chester,[36] Erwin Smith,[37] the Winslows,[38] Buchanan,[39] and Breed and Conn.[40] Chester adopted Migula's scheme of classification for the majority of organisms by morphology and flagellation, but put the more complicated forms like the tubercle bacillus and the diphtheria bacillus in the single genus Mycobacterium, discarding the genus Corynebacterium of Lehmann and Neumann. He further separated the genus Streptothrix from the Chalmydobacteriaceæ and restored the genus Leptothrix for the unbranched filamentous organisms. In 1905 Erwin Smith, in the first volume of his treatise on plant bacteriology, took up in great detail the various points at issue in taxonomy. He adopted Migula's arrangement of the bacteria in general, substituting the genus Bacterium Cohn with emendations for Pseudomonas Migula as a result of a careful study of Cohn's original paper and a repetition of his experiments. For the non-motile rod-shaped organisms of the type of the anthrax bacillus Smith proposed a new generic name, Aplanobacter (rod-without-motion). Non-sporulating, non-motile rods were included provisionally in the genus Aplanobacter. Smith regarded the genus Aplanobacter as identical with Bacteridium Davaine, Bacillus Cohn and Fischer, and Bacterium Migula. He followed Chester in including both the tubercle bacillus and the diphtheria bacillus in the genus Mycobacterium. In the treatment of the spiral organisms Smith followed Lafar, Fischer, Lehmann and Neumann for the comma-shaped spirals of the type found in Asiatic cholera, using the genus Vibrio Müller, emended by Cohn, and discarding the genus Microspira of Migula. In 1908 C-E. A. Winslow and A. R. Winslow suggested a revision of the Coccaceæ, chiefly on the basis of Gram's stain, pigment production, and parasitic or saprophytic growth. They divided the cocci into Gram-positive Paracoccaceæ which included most of the pathogenic species, and Metococcaceæ which included the saprophytic species. Buchanan has made a thorough historical investigation of nearly all the different groups of the simpler bacteria and Breed and Conn have cleared up many doubtful points in the group Actinomyces.

The most ambitious attempt to establish a consistent system of classifying the bacteria on the basis of our present knowledge of their morphology, physiological properties, cultural characters and pathogenic action is that of the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types, composed of Winslow, Broadhurst, Buchanan, Krumwiede, Rogers, and Smith. This Committee has now issued two reports covering their work.[41] They have examined previous classifications with great care and have attempted to determine the exact significance of the terms employed for families and genera. They

find 38 well-defined groups of bacteria, some of these marked by morphological characters, some by physiological properties and some by pathogenic action. These 38 groups have been arranged in orders, families, tribes, and genera according to the Linnæan system and the principles of botanical nomenclature.

The important point in which the scheme of classification presented by this committee differs from preceding schemes is their more complete consideration of the many non-pathogenic forms not included previously, such as the soil bacteria, etc., their utilization of staining reactions, pigment production and certain physiological characters, such as the fixation of nitrogen, for the establishment of genera.

A few of the important changes recommended by the committee may be mentioned. The genera *Mycobacterium* and *Corynebacterium* of Lehmann and Neumann are included in the family *Mycobacteriaceæ* of Chester together with other genera, the *Pseudomonas* is put into a special family, Pseudomonadaceæ which is separated from other organisms, only two genera of spiral formed organisms are accepted, Vibrio and Spirillum; the Gram-negative diplococci are put together in the Neissereæ, the genus Staphylococcus is restored for the Gram-positive pathogenic cocci, and a group Micrococcus established for the Gram-negative cocci of environmental origin. The organisms producing a red pigment are placed in special genera, Rhodococcus for the spherical organisms and Erythrobacillus for the rod-shaped forms. The Gram-positive Proteus forms are put in a new tribe, Zopfeæ, the Gram-negative Proteus forms in a genus, Proteus, under the Bacteriæ along with the non-sporulating forms of the genus Bacterium.

The lactic acid bacilli are regarded as a special tribe, Lactobacillaceæ, with one genus Lactobacillus; the organisms of the hemorrhagic septicemia group put in the genus Pasteurella in the tribe, Pasteurelleæ, the hemoglobinophilic organisms in a new genus, Hemophilus, in a new tribe, Hemophileæ. Finally the aërobic sporulating rod-shaped organisms are placed in the genus Bacillus, thus restoring spore formation to its proper place in classification. Another genus, Clostridium, is established for the sporulating anaërobes, and both these genera are included in the family Bacillaceæ.

Since the publication of the scheme of classification suggested tentatively by the Committee of the Society of American Bacteriologists, its value for American bacteriologists has been amply demonstrated. As far as possible this classification will be adopted in this book with some additions, as a basis for the separation of the bacteria into their primary groups. No attempt will be made to change all the names now in common use for the different genera and species since many of these names are so firmly entrenched in the literature of bacteriology that considerable confusion will result if they are discarded at the present time. The further details of the classification will be discussed in the special chapters devoted to systematic bacteriology.

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# PART II

# SYSTEMATIC BACTERIOLOGY

## Outline of Bacterial Classification

## CLASS SCHIZOMYCETES

Minute, one-celled, chlorophyll-free, colorless, rarely violet-red or green-colored plants, which typically multiply by dividing in one, two, or three directions of space. The cells thus formed are usually spherical, cylindrical, comma-shaped, spiral, or filamentous and are often united into filamentous, flat, or cubical aggregates. Filamentous species often surrounded by a common sheath. The cell plasma generally homogeneous without a morphologically differentiated nucleus. Reproduction by simple fission. In many species resting bodies are produced, either endospores or conidia. Cells may be motile by means of flagella.

### A. ORDER MYXOBACTERIALES*

Cells united during the vegetative stage into a pseudoplasmodium which passes over into a highly-developed cyst-producing resting stage.

### B. ORDER THIOBACTERIALES

Cells free or united in elongated filaments. Typically water forms, not cultivable on ordinary media. Life energy derived mainly from oxidative processes. Cells typically containing either granules of free sulphur or bacterio-purpurin or both, usually growing best in the presence of hydrogen sulphid.

#### Thiobacillus Beijerinck

Small rod-shaped organisms deriving their energy from the oxidation of sulphids, thiosulphates or elementary sulphur, forming sulphur, persulphates, and sulphates under acid or alkaline conditions and deriving their carbon from carbon dioxid or from bicarbonates and carbonates in solution.†

Type species *Thiobacillus thioparus* Beijerinck.
" *denitrificans* Beijerinck.
" *thiooxidans* Waksman and Joffe.

#### Thiobacillus thioparus Beijerinck

Isolated by Beijerinck[1] in a nutrient solution containing sodium bicarbonate as the only source of nitrogen and sodium thiosulphate, $Na_2S_2O_3$, as the source of energy, whose composition is as follows:

| | |
|---|---|
| $H_2O$, | 10.0 |
| $Na_2S_2O_85H_2O$, | 0.5 |
| $NaHCO_3$, | 0.1 |
| $K_2HPO_4$, | 0.02 |
| $NH_4Cl$ | 0.01 |
| $MgCl_2$ | 0.01 |

* Not treated in detail.
† Bergey: Manual of Determinative Bacteriology. 1923, p. 37.

Boiling or sterilization not necessary.

This solution is infected with a considerable quantity of ditch water or canal water or with a trace of ditch mud and then kept in the thermostat at 28° or 30° C., either in the light or dark. After two to three days the surface of the fluid is covered with a layer of free sulphur which is teeming with bacteria. By transferring material from this superficial film to fresh solutions a more abundant growth of the characteristic organisms may be obtained and eventually a pure culture. According to Natanssohn[2] and Beijerinck the splitting of the sodium thiosulphate takes place according to the following reaction:

$$S_2O_3Na_2 + O = Na_2SO_4 + S$$

This process is exothermic and can serve as a source of energy.

The organisms are small short slender rods measuring 0.5 x 3 m., very actively motile and containing no spores.

When cultivated on the nutrient solution to which 2 per cent. agar was added the organism produced small round colonies resembling dry yellow dust which died out in a week or less. A similar species was obtained from salt water mud and cultivated on the same nutrient solution containing 2 per cent. agar and 3 per cent. NaCl.

1. Beijerinck: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1904, xi, 593.
2. Natanssohn: Mitth. d. zoöl. Stat. zu Neapel., 1903, xv, 655.

### THIOBACILLUS DENITRIFICANS Beijerinck

Obtained by Beijerinck in a solution containing pure sulphur which functions as a source of energy by giving rise to the decomposition of carbonic acid. Subsequently denitrification takes place with the production of sulphates.

This solution has the following composition:

| | |
|---|---|
| Ditch water, | 100 |
| Sulphur as powder, | 10 |
| $KNO_3$, | 0.05 |
| $Na_2CO_3$, | 0.02 |
| $CaCO_3$, | 2 |
| $K_2HPO_4$, | 0.02 |

After five to six days at 30° C. the characteristic bacteria develop and may be cultivated by transferring to a similar solution made up with distilled water whose composition is as follows:

| | |
|---|---|
| $H_2O$, | 100 |
| Sulphur, | 10 |
| $KNO_3$, | 0.05 |
| $NaCO_3$, | 0.02 |
| $CaCO_3$, | 2 |
| $K_2HPO_4$, | 0.02 |
| $MgCl_2$, | 0.01 |

The reaction takes place by the following equation:

$$6KNO_3 + 5S + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 3N_2$$

Pure cultures were obtained on a medium containing agar of the following composition:

| | |
|---|---|
| Tap water, | 100 |
| $Na_2S_2O_3.5H_2O$, | 0.5 |
| $K_2HPO_4$, | 0.01 |
| $NaHCO_3$, | 0.02 |
| Agar, | 2 |

The colonies are large, thin, almost glass clear, difficult to see, and filled with drops of sulphur. They die out rapidly on plates. Cultivated also on nutrient gelatin of double strength containing 0.25 gram of $Na_2S_2O_3$. The colonies on this medium are small, round, and contain drops of sulphur.

Morphologically *Thiobacillus denitrificans* is a short rod actively motile and difficult to differentiate from *Thiobacillus thioparus*.

Beijerinck: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1904, xi, 593.

### THIOBACILLUS THIOOXIDANS Waksman and Joffe

An organism oxidizing sulphur to sulphuric acid isolated from the soil by Waksman and Joffe by inoculating composts of sulphur, rock phosphates, and soil into a medium of the following composition:

| | | |
|---|---|---|
| $(NH_4)_2SO_4$, | 2 | grams |
| $K_2HPO_4$, | 1 | gram |
| $MgSO_4$, | 0.50 | gram |
| KCl, | 0.50 | gram |
| $FeSO_4$, | 0.01 | gram |
| Sulphur, | 10 | grams |
| $Ca_3(PO_4)_2$, | 2.50 | grams |
| Distilled water, | 1000 | c.c. |

The medium is distributed in 100 c.c. portions into 250 c.c. Erlenmeyer flasks and sterilized by streaming steam, for thirty minutes, on three consecutive days. After inoculation with 1 c.c. quantities of varying dilutions of the compost the flasks become turbid in four to five days and are found to contain a minute non-motile bacterium together with fungi. A pure culture is obtained by continued transfer to fresh flasks in high dilutions, the medium being made acid at the start ($p$H 2–3) by the use of phosphoric acid and monopotassium phosphate.

**Morphology.**—Short rods with rounded ends occurring singly, to some extent in pairs, and rarely in triplets. Majority less than 1 $\mu$ long and about 0.5 $\mu$ in diameter. No spore-formation. Usually non-motile, although a few motile cells can also be found in young (seven-day) cultures.

**Staining Reactions.**—Stains well with gentian-violet and methylene-blue. Gram-positive.

**Cultivation.**—No growth on agar or other solid medium. Growth in liquid media with a strong uniform clouding without surface growth or sediment formation. No growth on common organic media. Best cultivated in inorganic media containing sulphur as a source of energy. In the presence of tricalcium phosphate the growth of the organism is accompanied by characteristic reactions; the sulphur forming originally a layer on the surface of the medium usually drops to the bottom, the sulphuric acid formed from the oxidation of the sulphur dissolves the tricalcium phosphate giving soluble phosphate and $CaSO_4.2H_2O$; the calcium sulphate crystallizes out in the form of radiating monoclinic crystals hanging down from the sulphur particles that are floating on the surface of the medium or protuding upward from the bottom. The reaction of the medium becomes acid as indicated by the change in the hydrogen-ion concentration. At a $p$H of about 2.8, the reaction becomes stationary till all the calcium phosphate has been dissolved. The culture is injuriously affected by an excess of this neutralizing agent, the presence of rapidly dissolving alkaline carbonates, by anything that changes the medium to an acid or less alkaline reaction (except the action of the buffers). The culture can be kept alive and active for numerous consecutive generations on the proper liquid media. This organism derives its carbon from the $CO_2$ of the atmosphere, its energy from the oxidation of sulphur, although it may also utilize thiosulphate. Hydrogen sulphid and sulphids are not utilized at all. Its mineral requirements are supplied by mere traces of K., Mg., Ca, and Fe. Its nitrogen is derived from the contamination of other salts or those distilled with nitrogen compounds, or from the ammonia of the atmosphere. The best sources of nitrogen are ammonium salts of inorganic compounds, ammonium salts of organic acids, nitrates, asparagin, and amino-acids. The organ-

ism is aërobic, deriving the oxygen necessary for the oxidation of sulphur to sulphuric acid from the atmosphere.

Waksman and Joffe: J. Bact., 1922, vii, 239.

## C. ORDER CHLAMYDOBACTERIALES*

Cells normally united in elongated filaments, often showing false but never true branching. Typically water forms. Sulphur and bacteriopurpurin are absent. Iron often present and usually a well-marked sheath.

## D. ORDER ACTINOMYCETALES Buchanan

Cells usually elongated, frequently filamentous, and with a decided tendency to the development of branches, in some genera giving rise to the formation of a definite branched mycelium. Cells frequently show swellings, clubbed or irregular shapes. No pseudoplasmodium. No deposits of free sulphur or iron. No bacteriopurpurin. Endospores not produced, but conidia developed in some genera. Usually Gram-positive. Non-motile. Some species are parasitic in animals or plants. No water forms. Complex proteins frequently required. As a rule strongly aërobic (except for some species of Actinomyces and the genera Fusiformis and Leptotrichia) and oxidative. Growth on culture media often slow; some genera show mold-like colonies.

### Family I. Actinomycetaceae Buchanan

Filamentous forms often branched and sometimes forming mycelia. Conidia sometimes present. Some species parasitic.

#### Genus 1. Actinobacillus Brumpt

Filament formation, resembling streptobacilli. In lesions no mycelium formed, but at peripheries finger-shaped branched cells are visible. Gram-negative. Not acid-fast.

Type species, *Actinobacillus Lignièresi* Brumpt.
*Bacillus actinoides* Smith.

##### Actinobacillus Lignièresi Brumpt

Isolated by Lignières and Spitz[1] from a peculiar lesion in animals which they regarded as related to true actinomyces. The pus in these cases contained very small mucus or calcareous grains consisting of central granular portions surrounded by radiating filaments. They gave the same histochemical reactions as the grains in true actinomycosis. That is they retained acid coloring substances, especially picric acid. The filaments showed no striations but frequent budding. On staining with Gram the central part of the grains decolorized and the whole mass of filaments took the counterstain, eosin or safranin. None of the filaments showed true branching.

The micro-organism isolated from these lesions was named by Brumpt.[2] The observations of Lignières and Spitz were confirmed by Nocard[3] and by Higgins.[4]

* Not treated in detail.

**Morphology.**—Small bacilli a trifle larger than the organisms of chicken-cholera. In young cultures usually bacillary, later, especially on agar, appearing as a coccobacillus or a diplococcus. In serum broth it appears as a streptobacillus. In old cultures, especially in broth containing agglutinating serum, bizarre involution forms occur. The bacillary forms measure about 0.4 by 1.15 to 1.25 $\mu$. Non-motile. No spores.

**Stains** easily by the usual anilin dyes, especially carbolfuchsin and acid violet. Gram-negative. Bipolar staining marked so that the organisms resemble the bacteria of hemorrhagic septicemia.

**Cultural Characters.**—Growth on the usual culture media as a facultative anaërobe. Turbidity and a powdery sediment in broth. No change in reaction. No odor. More abundant growth in serum broth. Acid reaction in glucose or lactose broth. Acidity in milk. No coagulation. Later the milk turns greenish as if it were alkaline. Small white colonies on gelatin without liquefaction. Small translucent bluish or opaque colonies on agar. Fairly abundant expansion on agar slants. Whitish expansion on coagulated serum. Scanty grayish-yellow growth on alkaline potato. Indol positive. Destroyed by heating one and one-half hours at 52° C., one hour at 54° C., ten minutes at 62° C., and one minute at 100° C.

**Pathogenic Action.**—Virulent to the usual laboratory animals such as rabbits, cats, and dogs. Subcutaneous inoculation gives rise to abscesses. Intraperitoneal inoculation in rabbits produces nodules filled with pus containing the organism but no "grains." In guinea-pigs intraperitoneal inoculation gives rise to purulent nodular foci containing bunches of characteristic clubs. Subcutaneous inoculation in sheep and cattle produces chronic abscesses which contain bunches of characteristic clubs identical with those in the natural disease.

1. Lignières and Spitz: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1904, xxxv, 294; 452.
2. Brumpt: Précis de parasitologie. Paris, 1910, p. 849.
3. Nocard: Les maladies microbiennes des animaux. 3. ed. Paris, 1903, ii, 374.
4. Higgins: Actinobacillosis, Bull. No. 1, Dept. Agr., Health of Animals Branch, Dominion of Canada, 1904.

### Bacillus actinoides Smith

Described originally by Smith from an epizoötic of pneumonia in calves. Subsequently found in both sporadic and epizoötic cases.

**Morphology and Methods of Isolation.**—Obtained from infected tissues most easily by the use of coagulated blood-serum in tubes sealed with sealing wax. After several days minute whitish flocculi are visible in the condensation water by transmitted light. The flocculi vary from minute specks to masses about 1 mm. in diameter. Under low power these flocculi appear made up on the periphery of radiating sheaths or filaments ending in clubs, together with minute irregular masses of crystals, and cylindrical filaments about 10 mm. in length not ending in clubs. If flocculi two to four days old be dried on coverslips and then fixed and stained with anilin dyes, such as alkaline methylene-blue, for several hours and finally decolorized in 0.01 per cent. acetic acid, they reveal the structure. The flake remains unstained and the interior is occupied by a tangle of very fine segmented filaments made up of bacilli. These show no evidence of branching and measure 0.4 to 0.5 $\mu$ in diameter. They stain in alkaline methylene-blue and dilute carbolfuchsin and are decolorized by Gram.

Such sealed cultures on horse serum may be maintained for many generations but the growth is improved when about 1 c.c. of calf-serum-water is added to the water of condensation. No multiplication occurs in open tubes. In subcultures after several weeks at 37° C. or at room temperature the slanted surface may become covered with very minute, elevated pointed-like colonies which microscopically have the same structure as the flocculi in the condensation water. In cultures three to four days old the flocculi now take up some of the stain and appear striated or lamellated as if some substance were deposited within the membrane. Peculiar bizarre forms appear which may be compared with the myelinic structure of nerve tissue or the so-called Buhl's bodies seen in fresh unstained sputum from the alveolar epithelium of the lungs. Bacteria which are present within the flakes in early vigorous stages no longer appear, being dissolved or being no longer accessbile to the stain.

After several months of cultivation further changes occur in the flocculi which are presumably degenerative in character. The clubs become surrounded with a layer of fine granules and are eventually concealed by them. These granules are slightly refringent, variable in size, and may be 1 mm. in diameter. They become detached from the flakes and collect in small groups at the surface of the condensation water as a white creamy layer, suggesting some fatty substance. They may be tinted with Scharlach R, but cannot be definitely regarded as fat.

After serum cultures are established and subcultures regularly successful the organism

can occasionally be transferred to sealed slant agar, especially if this contains a bit of guinea-pig spleen and some calf-serum-water is added to the condensation water. The colonies are very minute, drop-like, discrete or larger, up to 1 to 1.5 mm. in diameter. The growth usually starts from the condensation water and extends 5 to 10 mm. up on the slant. The colonies are straw-colored, coherent, and can be removed from the agar slant "in toto." Microscopically the colonies consist of bacilli, close together and with no indication of capsular substance. After one to two weeks' growth on agar slants a thin creamy layer may appear on the condensation water. The growth meantime shows a disappearance of the bacillus type of organism and an appearance of coccus types like those seen on agar slants from diseased lung tissue.

Growth takes place also on sealed slant agar to which particles of diseased lung are added, either as a film covering the surface of the water of condensation or as roundish slightly raised yellowish colonies 1 to 2 mm. in diameter. Such colonies consist microscopically of roundish ring-like rather faint bodies about 2 $\mu$ in diameter with a very minute refringent speck either in the center or at the periphery. This appearance suggests spore-formation. Subcultures from such growths are not successful. The organism cannot be cultivated on ordinary slant agar, milk, potato, broth plus tissue, ascitic fluid plus tissue, or fermentation tubes plus tissue.

Sheaths or capsules are absent in agar slant cultures with blood or tissue, in agar with milk, with or without the fat. They are also absent on coagulated blood-serum. Both the sheathed and the unsheathed varieties of the organisms may be obtained from the same case and grown in pure culture indefinitely. When capsules are not produced the growth on the surface of the serum is very feeble and the chalky whiteness is replaced by a colorless semi-translucent appearance due to the point-like colonies. In the water of condensation the floccules are replaced by a fine powdery cloudiness due to small groups and clusters of bacilli.

**Pathogenic Action.**—No lesions produced in small animals. Subcutaneous inoculation in calves produces a large swelling which becomes necrotic with caseous contents and a thick firm wall of hyperplastic connective tissue. The capsule softens and the overlying skin ulcerates, an outward discharge taking place in about four weeks. Inoculations in the tissues about the trachea in calves also lead to large abscesses made up of a soft mass like cottage cheese. Microscopically this shows an increase of mononuclear and endothelial cells which are later invaded by polymorphonuclear cells. The bacilli are present in abundant clouds in the zones outside the necrotic masses. Direct injection into the trachea produces small necrotic foci in the lungs identical with those in the natural disease.

This organism is regarded by Smith as the primary inciting agent of bronchopneumonia in calves, other organisms like *Bacillus pyogenes* and *Bacillus bovisepticus* later growing in the occluded air tubes and necrotic tissue. It is not the actinobacillus of Lignières and Spitz which produces abscesses but not pneumonia.

Smith: J. Exper. M., 1918, xxviii, 333. *Ibid.*, 1921, xxxiii, 441. *Ibid.*, 1921, xxxiv, 593.

## GENUS 2. LEPTOTRICHIA Trevisan

Thick, long, straight, or curved threads, unbranched, frequently clubbed at one end and tapering to the other. Gram-positive when young. Threads fragment into short, thick rods. Anaërobic or facultative. Non-motile. Filaments sometimes granular. No aerial hyphæ or conidia. Parasites or facultative parasites.

Type species *Leptotrichia buccalis* (Robin) Trevisan. While this genus is properly called Leptotrichia the more familiar term "Leptothrix," by which the organisms were described, may be retained for the present. The following are the most important species:

*Leptothrix buccalis* (Robin) Trevisan.
" *epidermidis* Bizzozero.
" *innominata* Miller.
" *vaginalis* von Herff.
" *pyogenes filiformis* Flexner.
" *placoides alba* Dobrzynicki.
" from urine of Arustamow.
" from pharynx of Arustamow.
" Parinaud's conjunctivitis of Verhoeff.
" in hemorrhagic nephritis of Dick and Rappaport.

*Leptothrix* in bronchopneumonia of Dick.
" in conjunctiva and meibomian glands of Gifford.

### LEPTOTHRIX BUCCALIS (Robin) Trevisan

A long recognized saprophite of the buccal cavity. Doubtful if it possesses pathogenic properties or has been cultivated.

Described in pharyngitis by Fraenkel[1] (benign mycosis of the pharynx).

Vignal[2] claimed to have obtained pure cultures of *Leptothrix buccalis*. Not confirmed.

Cases observed also by Chiari,[3] Michelson, and Epstein.[4]

1. Fraenkel: Schlundkopf, Eulenberg's Realenzyklopädie der gesamten Heilkunde. 1882.
2. Vignal: Arch. de physiol. norm. et path., 1886, 3. s., viii, 325.
3. Chiari: Revue mensuelle de laryngologie, d'otologie et de rhinologie, 1887, No. 10.
4. Epstein: Prag. med. Wchnschr., 1900.

### LEPTOTHRIX EPIDERMIDIS Bizzozero

Described originally by Bizzozero[1] from the skin of healthy men.

Cultural characters of a similar leptothrix worked out in detail by Lehmann and Neumann,[2] but it is not certain that it was Bizzozero's organism.

**Morphology.**—Fairly thick threads growing in chains, easily separated, without branching. Young cultures show bacilli like *Bacillus mesentericus*. No spores.

**Motility** observed in young rods. Flagella not stained.

**Staining Reactions.**—Stains with ordinary anilin dyes. Gram-positive. The organisms do not stain blue with iodin and potassium iodid.

Growth better under aërobic than under anaërobic conditions. Luxuriant growth at room and body temperature.

**Cultural Characters.**—*Gelatin Plates.*—Minute white points which liquefy the gelatin in twenty-four to forty-eight hours. Older colonies show small white flakes in the center of the liquefied areas which show a whitish border. Under low power (50 diameters) the young superficial colonies appear as convolutions of curly wavy threads. The centers become dark and cloudy, sunken, surrounded peripherally by a row of fine radiating hairs. With more advanced liquefaction a flat grayish saucer develops which presents a delicate hairy border toward the solid gelatin, with a curly mass in the middle.

*Gelatin Stab.*—Conical area of liquefaction in twenty-four hours with whitish flocculi and a deposit of yellowish-white masses of bacteria. After four to five days a grayish tough pellicle on the surface.

*Agar Plates.*—Superficial colonies white or yellowish-white, sharply outlined, with smooth irregularly notched borders. Deep colonies small dense and yellowish-white. Under low power (50 diameters) the superficial colonies have opaque brownish-yellow centers, passing gradually into a yellowish-gray peripheral zone which consists of very closely-set fine radiating hairs. Deep colonies roundish, nodular, yellowish-brown, here and there presenting single or bunched projecting hairs.

*Agar Stab.*—Yellow-gray to brown surface growth, smooth-bordered and slimy. It gradually becomes dull with whitish elevations. Grayish-white thread along the stab. After several months the agar turns dark brown.

*Agar Slant.*—Yellowish-gray to brown smooth-bordered slimy growth, with a tough wrinkled brownish pellicle on the water of condensation along the wall of the tube. This pellicle is pure white. Water of condensation remains clear.

*Broth.*—Tough thick wrinkled surface pellicle, firmly adherent to the wall.

*Milk.*—Dense scum on the surface. No coagulation. The milk becomes transparent and a limited white precipitate is formed.

*Potato.*—Rapid growth, elevated, reddish to grayish-brown, sharply outlined. The surface growth develops many wrinkles. In old cultures the peripheral zone presents a white chalky discoloration.

1. Markoff: [Abstr.] Centralbl. f. Bakteriol. [etc.], 1896, xx, 604.
2. Lehmann and Neumann: Atlas and Principles of Bacteriology. 1901, p. 478.

### LEPTOTHRIX INNOMINATA Miller

Reported originally by Miller[1] in the mouth. Cultivated successfully once by Wherry and Oliver[2] on Martin's medium[3] with reactions varying from +0.5 to +2, the medium containing an equal quantity of ascites or pleural fluid that had been heated to 56° C. for one hour on five to six successive days. The organism was adapted to a wide range of oxygen tension and is regarded by Wherry and Oliver as a microserophilic anaërobe. After once growing away from human tissues it thrives best on beef-extract agar containing some suitable carbohydrate. Description from Wherry and Oliver.

**Morphology.**—Usually in twos, threes, and chains of seven or more segments. Each segment is an elongated rod, usually with rounded ends, although terminal segments may exhibit a tapering, somewhat pointed free end. When unstained the cells are 1.4 $\mu$ in diameter, when fixed with methyl alcohol and stained with dilute carbolfuchsin they are 0.84 $\mu$ in diameter. Single segments vary from 5 to 14 $\mu$ in length. The segments are attached to each other in a straight line and seem to be covered by a common delicate envelope. Long chains of segments occur, as in milk where they may be 336 $\mu$ long, bend like a hair and not like a chain of loosely joined rods. Cytoplasm fairly homogeneous but may show a fine vacuolization along the central longitudinal axis. In cultures a week old on media containing fermentable carbohydrates the cytoplasm is markedly vacuolated and the stained cells show many small irregularly distributed and deeply stained areas. No bodies resembling spores seen. No motility. Anaërobic cultures on +1 maltose agar after eight days at 37° C. show a number of coiled forms. They are apparently coiled like a corkscrew at one end, then bent over and grown around the lengthening filament, thus resembling a twisted loop of rope.

**Staining Reactions.**—The organisms when fixed by heat or methyl alcohol stain readily with dilute carbolfuchsin, Bordet's carbol toluidin blue, or Nicolle's carbol gentian-violet. They stain faintly with Loeffler's methylene-blue in five minutes. Gram-negative. *Granulose reaction.* The organism usually takes a yellow color in Gram's iodin. When grown on +1 maltose for six days at 37° C. about 1 per cent. give a marked reaction, the granulose being present in large blocks with intervening colorless segments.

**Cultural Reactions.**—Isolated by Wherry and Oliver from scrapings from the gums on the modifications of Martin's medium solidified in Petri dishes. The best modification proved to be one containing 1 per cent. sodium sulphite and almost an equal volume of sterile human pleuritic fluid (heated to 56° C. for five successive days). The plates were kept at 37° C. under anaërobic conditions by pyrogallic acid and caustic by Lentz's method.[4] The colonies first appeared as a loose thatch-like mass which grew and became visible to the naked eye in four to five days. When transplanted to slants of the same medium, the growth was much more luxuriant, isolated colonies reaching a diameter of from 0.5 to 3 mm. in a few days. They presented a fairly characteristic appearance when viewed through a hand-lens, that is they appeared like Medusa-lock colonies with a pearly iridescence. (Such colonies are also found with *Bacillus coli* and *Bacillus typhosus* grown under partial tension.)

Growth also on Martin's pleuritic medium under anaërobic conditions and under partial tension produced by *Bacillus subtilis*. Aërobic subcultures on the same medium remained sterile. Growth under anaërobic conditions fairly luxuriant on +1 maltose agar containing blood, where the medium as well as the growth assumed a brownish-red hue. Growth on +1 lactose agar. No growth on +1 agar, +1 dextrose agar, +1 glycerin agar, +1 broth, +1 dextrose broth or +1 Dunham's peptone made from Liebig's extract. In litmus milk growth took place with the production of acid without coagulation and the formation of a yellowish waxy mass which floated on the surface and resembled cerumen from the human ear. This was composed microscopically of coarse and fine fatty acid crystals. The leptothrices were found only in the sediment and some of them were very long, 336 $\mu$ in one case. They contained irregularly placed spherical refractile bodies of the same diameter as the filaments. They did not give the granulose reaction. Flocculent growth in pleuritic fluid added to potato. This was composed of normal forms but many filaments were present, showing irregular globular swellings and some free ovoid globular forms, 3.3 $\mu$ in diameter. None of these forms gave the granulose reaction.

*Fermentation Reactions.*—Media made in +5 Martin's medium in tubes to which were added a few drops of a 10 per cent. carbohydrate or alcohol solution in distilled water (previously sterilized at 15 pounds for fifteen minutes) and sufficient sterile litmus and pleuritic fluid. On such anaërobic slants there was marked acid production with precipitation of serum in dextrose, levulose, galactose, lactose, saccharose, maltose, inulin, dextrin, raffinose, and mannose. No action on mannite, erythrite, dulcite, and isodulcite.

1. Miller: Bakteriologie der Mundhöhle. 1894.
2. Wherry and Oliver: J. Infect. Dis., 1916, xix, 299.
3. Martin: J. Path. and Bacteriol., 1911, xv, 76.
4. Lentz: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1910, liii, orig., 358.

### LEPTOTHRIX VAGINALIS von Herff

Found by von Herff in a case of vaginal mycosis. Not cultivated.

von Herff: Ueber Scheidenmykosen (Colpitis mycotica acuta) Samml. klin. Vortr. n. F., 1895, No. 137.

### LEPTOTHRIX PYOGENES FILIFORMIS Flexner

Described by Flexner[1] in acute endometritis in a rabbit, accompanied by pleurisy, pericarditis, and pneumonia. Subsequently found by Muscatello.[2]

**Morphology.**—In the exudate in the disease the organism appears as felted networks of bacilli about 0.5 to 0.7 $\mu$ wide and varying in length, 1.4 to 154 $\mu$ representing the extremes. The average length is between 56 and 70 $\mu$. The long forms show delicate long and sinuous curves. They are usually extracellular being rarely taken up by phagocytes. The organisms stain irregularly and are composed of longer or shorter strands of protoplasm taking a very faint color with anilin dyes, in which lie at regular intervals deeply stained points of chromatin about equidistant from each other. They are cylindrical rather than globular, generally longer than the width of the protoplasm in which they lie. The short forms may contain 2 to 3 of the granules, the long forms as many as 100. No branching observed. Non-motile.

**Stained** by the usual anilin dyes, carbolfuchsin and Stirling's anilin oil gentian-violet. After treatment of the stained material with 1 : 1000 aqueous solution of glacial acetic acid the irregular staining is most apparent. Decolorized by Gram and Weigert's methods. If unheated material containing the living bacilli be treated with carbolfuchsin or gentian-violet, the chromatin points take the stain, leaving the protoplasmic mass but slightly tinted. The chromatin points are regarded as preformed in the bacterial cells.

**Pathogenic Action.**—Intrapleural inoculation with animal exudates in rabbits produces a shaggy yellowish pseudomembranous exudate with a multiplication of the organisms. The animals die in from twelve hours to five days. Sometimes the inflammatory process extends into the pericardium, produces small abscesses, or into the peritoneal cavity, producing pseudomembranes on the cephalic surface of the liver. In other instances patches of pneumonia are found in the lungs. In guinea-pigs intrapleural inoculation produces the same type of lesion, a sticky, thick, slightly adherent, greenish-white pseudomembrane. Animals also die from intraperitoneal inoculation, but not uniformly as with intrapleural injection. Death occurs in rabbits in forty-eight hours and at autopsy there is a sticky fluid containing large flakes of fibrin in the peritoneal cavity. The inflammation may also extend into the pleura setting up a characteristic pleurisy. In pregnant animals direct injection into the uterus produces an inflammation resulting in abortion and the disease process may extend into the peritoneal cavity. Intrameningeal inoculation produces fatal meningitis in rabbits. Fatal results also follow subcutaneous inoculation, an extensive yellowish-white, rather dry necrosis developing and involving a considerable area about the site of inoculation. Following intravenous inoculation of pleural exudate in rabbits the animals die in forty-eight hours with abscesses in the viscera. These are either miliary or larger and spreading. They are never absent from the brain and heart muscle, appear occasionally in the liver, rarely in the voluntary muscles, never in kidneys, spleen, or lungs. Finally intravenous inoculation is sometimes followed by general septicemia, the organisms being present in the blood and in all the organs.

**Cultivation.**—Attempts to cultivate the organism were made on a great variety of media, especially those containing serum from animals, the juice of macerated organs, etc. In no case was cultivation successful. Growth was observed for several generations on organs removed aseptically from the rabbit, especially the sterile fetus. Here the organisms were practically the same as in infected tissues except that they stained solidly, the granular forms found in the tissues not being present in the cultures. The organisms died out with some rapidity on the organs.

After heating to 55° C. for five minutes, the living organisms were deprived of virulence. The same effect followed drying at 37° C. for forty-eight hours and short exposure to diffuse daylight.

1. Flexner: J. Exper. M., 1896, i, 211.
2. Muscatello: Estratto dal volume per il xxv anno dell' insignamento chirurgico di Francesco Durantis nell' universitè di Roma.

### Leptothrix placoides alba Dobrzyniecki

Obtained by Dobrzyniecki from a root filling in a tooth, four years old. Regarded by the author as a distinct species.

**Morphology.**—Entangled threads without structure in hanging drops. Extremely long. By staining it is possible to see inside the threads long chains formed of straight rods, between which round spore-like bodies are visible. The threads form beautiful coils like a long bundle of hair. Non-motile.

**Stains** with carbolfuchsin and best with gentian-violet. Iodin reaction positive with a solution of iodin in potassium iodid slightly acidified with lactic acid. The segments take on a light violet color. Gram-positive.

**Cultivated** at temperatures between 16° and 18° C. Growth slow. The organisms have considerable vitality and can be transferred for a long time.

*Gelatin Plates.*—Round raised colonies in forty-eight hours, the size of oat kernels. Under low power seen to be made up of long threads like colonies of *Bacillus anthracis*. After three days liquefaction sets in. Colonies remain white and hard and swim about in the liquid gelatin so that the whole mass looks like gruel.

*Agar Plates.*—Colonies grow slowly and in small numbers, about half a lentil in size.

*Agar Slant.*—Characteristic growth after eight to ten days along the line of inoculation as a band made up of clear rosette-formed white colonies as hard as cartilage. They are visible as a series of diminutive scales. The whole colony can be raised with a platinum needle.

*Gelatin Stab.*—After four to five days isolated white colonies appear and after ten days the gelatin is softened about them.

*Blood-serum.*—Growth like that on agar. The serum is liquefied in old cultures.

*Potato.*—No growth.

*Broth.*—No growth.

Dobrzyniecki: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1897, xxi, 225.

### Arustamow's Leptothrix from urine

Isolated from urine by Arustamow as Leptothrix No. 1.

**Morphology.**—Homogeneous threads on potato, slightly curved, 0.5 to 0.6 $\mu$ thick and 8 to 50 $\mu$ long. In agar the threads are shorter and more curly, forming many tangles. In fluid media they are longer and less curving.

**Staining Reactions.**—Stains by the usual anilin dyes showing no dichotomous division, transverse division, or granulations. In old cultures the contours of the threads become less distinct and along their entire length glistening regularly placed spheres develop, the diameter of which is somewhat less than that of the threads. When such a thread is stained the sheath is only weakly colored while the spheres are intensely stained. If such old cultures are transferred one can follow all gradations from the spheres to long threads. The spheres are regarded as spores by Arustamow.

**Cultivated** only under anaërobic conditions, best at 37° C. usually below the surface of the media.

*Agar Plates.*—Very small transparent colonies barely visible to the naked eye. More abundant development on slightly acid media. Under low power colonies star-shaped, centers darker, consisting of a convolution of threads which extend radially toward the periphery.

*Agar Stab.*—Slight growth along entire line of stab as a homogeneous milky streak. Cultures retain vitality forty days.

*Broth and Urine.*—Faint clouding on the bottom of the tubes.

*Potato.*—Very slight development.

Arustamow: Zur Morphologie und Biologie der Leptothrix. Vrach, 1889, Nos. 3 and 4. Ref. by Besser. Centralbl. f. Bakteriol. [etc.], 1889, vi, 349.

### Arustamow's Leptothrix from the pharynx

Isolated by Arustamow from the tonsils in 2 cases of mycosis of the pharynx. Described as Leptothrix No. 2.

**Morphology.**—Like the leptothrix from the urine. Threads 0.5 to 0.6 $\mu$ thick, of variable length, usually homogeneous. Transverse division seen in some threads (explained by the author as a coalescence of several short threads). In old cultures granules develop like those in the urine leptothrix.

**Growth** aërobic, at 37° C., slight at room temperature, preferably on the surface.

*Agar Plate.*—Colonies like those of the urine leptothrix but much larger, star-shaped and transparent, the centers made up of a thick convolution of threads, forming a network, gradually disappearing toward the periphery.

*Agar Stab.*—Thick folded grayish membrane on the surface, very slight development along the stab.

*Gelatin.*—Slowly liquefied.

*Broth and Urine.*—White folded membranes covering the entire surface.

Arustamow: Zur Morphologie und Biologie der Leptothrix. Vrach, 1889, Nos. 3 and 4. Ref. by Besser. Centralbl. f. Bakteriol. [etc.], 1889, vi, 349.

### Verhoeff's Leptothrix from Parinaud's conjunctivitis

Leptothrix forms in Parinaud's conjunctivitis, which is characterized by unilateral development of nodules beneath the conjunctiva and involvement of the pre-auricular gland, were first described by Verhoeff.[1] The filaments were present in irregular masses, 10 to 16 $\mu$ in diameter, within or near the areas of cell necrosis beneath the epithelium. Individual filaments delicate, about the diameter of the influenza bacillus, varying from a few to 30 $\mu$ in length. Straight, irregularly curved, or bent. Stained poorly, best demonstrated by a modified Gram which brought out rather regularly distributed granules. No branching.

Subsequently Wherry and Ray[2] cultivated a leptothrix from a similar case of Parinaud's conjunctivitis. The pus from the pre-auricular gland was inoculated into the subcutis of a rabbit, guinea-pig, and white mouse. In the latter a vesicular swelling appeared at the site of inoculation, 4 mm. in diameter, followed by double purulent conjunctivitis. The mouse died on the twenty-first day. At autopsy muscular abscesses were found near the tendinous insertions of both knee-joints. Microscopically these showed minute polymorphous Gram-negative rods. They stained poorly with Loeffler's methylene-blue or carbol toluidin blue, best with steaming hot Giemsa solution.

The pus from these abscesses was inoculated on a variety of media and on coagulated egg-yolk slants, kept under anaërobic conditions and under partial tension. Numerous colonies appeared in forty-eight hours. These colonies were 0.25 mm. in diameter, raised, dryish, unpigmented. Microscopically they showed very delicate rods and filaments. The organism could not be transferred to ordinary media and died out after being kept three months at room temperature.

The organism was also cultivated directly from the pus of the pre-auricular gland on coagulated egg-yolk slants under partial tension. Fine colonies appeared after two weeks made up of minute polymorphous rods resembling those in the abscesses in the mouse. A mixture of pus from the eyes and muscular abscesses of the mouse was injected subcutaneously into another mouse which also developed double purulent conjunctivitis. This healed by the eighteenth day.

1. Verhoeff: Arch. Ophth., 1913, xlii, 345.
2. Wherry and Ray: J. Infect. Dis., 1918, xxii, 554.

### Dick and Rappaport's Leptothrix from chronic hemorrhagic nephritis

Isolated from the urine in a case of chronic hemorrhagic nephritis by Dick and Rappaport.

Small colonies in dextrose agar shake cultures of Gram-positive organisms, varying from short bacillary forms to long tangled threads, the thread form predominating. No true or false branching. No sheath.

Slow growth under anaërobic conditions.

Colonies on blood ascites agar transparent points, coalescing at the end of a week and forming a thin brownish opaque film.

Growth on human blood more luxuriant than on goat's blood and human ascites fluid agar. No change in the blood.

Little or no growth on ordinary media.

When first isolated large doses, whole agar slants, produced convulsions and death in rabbits.

Marked improvement in the patient by vaccine treatment.

Dick, Dick, and Rappaport: J. Infect. Dis., 1916, xviii, 216.

### Dick's Leptothrix from chronic bronchopneumonia

Obtained from the sputum in chronic bronchopneumonia by Dick on blood agar slants cultivated anaërobically. The growth showed many colonies of Gram-positive organisms growing in filaments as well as bacillary forms. The organisms were also found plentifully in direct smears of the sputum, which showed no tubercle bacilli. From pure cultures the organisms, when stained with carbolfuchsin and decolorized by 10 per cent. hydrochloric acid in 95 per cent. alcohol, were for the most part decolorized. Sections of the filaments and granules in the filaments retained the fuchsin so that smears counterstained with methylene-blue showed the long blue filaments with red dots and dashes spaced irregularly. Some of the short forms retained the fuchsin while others did not. In direct smears of the sputum the organisms were non-acid fast.

The strain grew fairly well on anaërobic blood agar cultures. In five to six days colonies appeared as small translucent flat disks reaching a maximum of 1 mm. in diameter. In subsequent transfers growth appeared at the end of forty-eight hours and at the end of five to six days the blood agar had become brown. In anaërobic dextrose-ascites-broth the organism tended to form colonies which sank to the bottom of the medium. No growth on ordinary media. No anaërobic growth. No branching.

Skin tests with the patient were positive, 0.1 c.c. of a suspension of 500,000,000 organisms per cubic centimeter injected intracutaneously producing redness and induration which was well-marked from twenty-four to forty-eight hours following the injection and subsided in about three days.

Rabbits injected intravenously either died over night from large doses or were not affected at all. No experimental pulmonary infection was obtained.

Dick: J. Infect. Dis., 1917, xxi, 9.

### Gifford's Leptothrix from conjunctiva and meibomian glands

Gifford has reported the finding of leptothrix forms in the conjunctiva and in the secretions from the meibomian glands. He differentiates three strains.

No. 1. Conjunctival secretions showed numerous Gram-positive bacilli, many 10 to 15 $\mu$ long by 1 to $1\frac{1}{4}$ $\mu$ thick. Some organisms curved. Many presented clear refractile subterminal bodies which appeared to be spores. Organisms all extracellular. On blood-serum in twenty-four hours a pure culture of a Gram-positive organism developed long unbranched curved threads with some shorter rods. Non-motile.

In cultures a few days old, coils of the thread-like organism were found 50 to 100 $\mu$ long. Spores were found in forty-eight hours, seen subterminally on the rods and all along the threads and taking Moeller's stain. Some free spores. No branching. Serum liquefied in seven to ten days. Agar growth at 37° C. little at room temperature. Growth spreading, grayish-white in twenty-four hours with a slightly feathery edge. No pigment. The growth became constricted and roughened but never folded. Cultures made from single colonies showed both short rods and threads.

*Broth* showed a characteristic growth of feathery clumps at the bottom, sending upward long threads that almost reached the surface after ten days or more.

*Milk.*—No acid. Decolorized in forty-eight hours. No coagulation. Later peptonized.

*Potato* slow invisible slightly moist growth.

*Gelatin* liquefied slowly around the upper part of the stab at room temperature.

*No odor. No gas in sugar broth.*

Iodin reaction uniformly negative.

Spores most numerous on solid media. Longest threads found in liquid media. No anaërobic growth.

Not destroyed in a boiling water-bath in one hour. Cultures left at room temperature viable after several months.

**Pathogenic Action.**—Pathogenic to guinea-pigs, 5 minims of washings from an agar slant injected intraperitoneally killing in about eighteen hours, the organism being recovered from the peritoneal exudate but not from the heart blood. Two minims injected beneath the conjunctiva killed in twenty-three hours. No reaction was found in the orbital tissues but subcapsular hemorrhages were present in the kidneys and on the enlarged spleen. Smears and cultures were positive from the orbital tissues and heart's blood. Other guinea-pigs died five to ten days after injection of smaller quantities but the organism was not obtained at autopsy. Non-pathogenic to white mice. No effect on rabbits from subcutaneous inoculation. Rabbits immunized by intravenous inoculation had agglutinins for the organism in their blood-stream. Active up to a dilution of 1 : 640.

Apparently not injurious to the normal human conjunctiva.

No. 2. Whity waxy semifluid material obtained from the meibomian glands in a case of eye-strain and congestion of the conjunctiva showed a fair number of large bacilli with rounded ends but no true threads. Some organisms were Gram-positive, some Gram-negative. A pure culture was obtained from a colony on a plate. This showed after two to four days, many threads 10 to 20 $\mu$ long, some 20 to 50 $\mu$ long. Some developed clubbed extremities and produced subterminal spores which were stained by Moeller's method. Non-motile. No branching.

*Agar.*—At 37° C. a delicate grayish film spreading over the medium, not wrinkled.

*Milk.*—Slight acidity. No coagulation.

*Broth.*—No pellicle.

*Blood-serum.*—No liquefaction.

No growth at room temperature. Longest threads in fluid media. Not killed over boiling water in a water-bath in twelve minutes but killed in twenty-four minutes.

Non-pathogenic.

Not agglutinated by the serum made from No. 1.

No. 3. Smears from the meibomian glands in a case of error of refraction showed a few curved Gram-positive rods, some 20 to 25 $\mu$ long with shorter Gram-positive rods, some of which were curved. No branching. The threads were 1 to $1\frac{1}{4}$ $\mu$ wide and showed refractile spore-like bodies like those seen in Case 1. Not cultivated.

Gifford: J. Infect. Dis., 1920, xxvii, 296.

## GENUS 3. ACTINOMYCES Harz

Organisms growing in form of a much-branched mycelium, which may break up into segments that function as conidia. Sometimes parasitic, with clubbed ends of radiating threads conspicuous in lesions in animal body. Some species are micro-aërophilic or anaërobic. Non-motile.

The **actinomyces group** includes a large number of highly diversified organisms which have been known at various times as actinomyces, streptothrix, cladothrix, nocardia, oöspora, etc. The term "actinomyces" was originally employed by Harz for the organism found by Bollinger in a disease of cattle which exhibited peculiar concretions (Drusen) in which branching filaments with club-shaped extremities arranged in radiating rays or spokes formed the main element. Considerable discussion has arisen as to the organism which was responsible for this condition but there seems to be no reasonable doubt that the anaërobic species originally isolated by Wolff and Israel and subsequently described in great detail by Wright is the etiological agent in true actinomycosis of cattle and in the human cases which show similar lesions. The organism isolated by Bostroem from a large series of human infections, the pus of which shows granules, is prob-

ably not the cause of true actinomyces of cattle or man. We know now that a great variety of infections develop in man from *aërobic* organisms which are in general of the same type as the true *Actinomyces bovis* of Wolff, Israel, and Wright. Such infections occur about the jaws, in the abdominal cavity, in the brain, and especially in the lungs. We may therefore properly regard Bostroem's aërobic organism as *Actinomyces hominis* and group with it other aërobic organisms, particularly those isolated by Berestnew, Foulerton, and others. In addition to these, other pathogenic species have been isolated and described by a number of authors, such as *Actinomyces asteroides*, *Actinomyces capræ*, *Actinomyces canis*, *Actinomyces maduræ*, etc. While the lesions found in man and animals and produced experimentally by those organisms are not absolutely identical with those produced by *Actinomyces bovis*, they present essentially similar histological processes and the organisms causing them may be regarded as true actinomyces.

Somewhat different branching organisms have been isolated from other lesions of great variety in man and animals. These lesions are only superficially similar to the lesions in true actinomycosis and the organisms found differ considerably in morphology and in life history. These organisms are often separated from the actinomyces and called "streptothrix" or "nocardia." As pointed out by Breed and Conn the names "streptothrix" and "nocardia" have no validity. At the same time more careful study of the organisms known as streptothrix, etc., reveals the fact that many of them are much like true actinomyces, producing tangled filaments and conidia in their life cycle. For the present therefore all the forms described as streptothrix or nocardia may be grouped under the general heading of actinomyces.

Finally large numbers of branching filamentous forms with true conidia have been isolated from the atmosphere and from the soil, a few being associated with diseases of plants. Some of these are pathogenic to animals. How far all these air organisms are to be grouped with the pathogenic actinomyces is somewhat debatable. For the present they may be included since they resemble them in morphology and life history.

Of special importance to the physician and public health official is the fact that a number of actinomyces are acid-fast and in certain stages are difficult to differentiate from the tubercle bacillus. This is especially important since acid-fast actinomyces are not infrequently associated with chronic disease of the bronchi and lungs and it seems probable that these cases were originally diagnosed as tuberculosis. In this connection it may be found that those peculiar cultures of the tubercle bacillus which show branching and club-shaped extremities are not true tubercle bacilli, but acid-fast actinomyces (streptothrices).

Finally, it has been pointed out by Drechsler that the organisms described as actinomyces differ in many important particulars from the true bacteria and should properly be put in the group of *Hyphomycetes*.

## ACTINOMYCES

A. *Pathogenic.*

I. *Anaërobic.*

Type Species *Actinomyces bovis* Harz.
" *canis* Levy.
" *interproximalis* (Fennel).
Mayer's actinomyces.
Neschczadimenko's actinomyces.
Plaut's anaërobic actinomyces.

II. *Aërobic.*

Type Species *Actinomyces hominis* Bostroem.
" *Hoffmanni* Gruber.
" *liquefaciens* Garten.
" *maduræ* (Vincent).
" *farcinica* Nocard.
" *asteroides* (Eppinger).
" *lathridii* (Petruschky).
" *capræ* (Silberschmidt).
" of Sabrazès Rivière.
" *Gedanensis* (Scheele and Petruschky).
" *candida* (Gedanensis II) Petruschky).
" *Krausei* (Petruschky).
" *protea* Schurmayer.
" *Spitzi* Lignières and Spitz.
" *japonica* (Petruschky).
" of Birt and Leishman.
" *polychromogenes* (Vallée).
" of Zimmermann.
" *valvularis* Luginger.
" *Freeri* Musgrave and Clegg.
" *luteola* Foulerton.
" *variabilis* Cohn.
" *taraxeri cepapi* Schottmüller.
" *muris ratti* Schottmüller.
" of Bloomfield and Bayne-Jones.
" of Evans.
" *putorii* Dick and Tunnicliff.
" *cameli* (Mason).
" of Rodella.
" *gypsoides* Henrici and Gardner.

B. *Non-pathogenic.*

Type Species *Actinomyces Foersteri* (Cohn).
" *aurea* (Saint-Sévérin).
" *rubra* (Kruse).
" *Metchnikovi* (Sauvageau and Radais).
" *Guignardi* (Sauvageau and Radais).
" *invulnerabilis* (Kruse).
" *flava* Sanfelici.
" *chalcea* (Foulerton).
" of Caminiti.
" *violacea* (Kruse).
" *aurantiaca* (Kruse, 1896).
" *citrea* Gasperini.
" *carnea* Rossi-Doria.
" *chromogena* (Kruse).
" *albido-flava* (Kruse).
" *alba* (Rossi-Doria).
" *thermophilus* Gilbert.

### Actinomyces bovis Harz

The lesion known as actinomycosis, both in cattle and in man, is a suppurative process characterized by the formation of granules or "Drusen"

surrounded by zones or walls of granulation tissue. These granules microscopically are made up of dense masses of filamentous organisms which show true branching and peculiar club-shaped structures at the periphery of the granule, often regarded as degeneration products. Ever since the disease was first described by Bollinger[1] and the branching organisms found in it named by Harz,[2] attempts have been made to cultivate them. The first positive results were probably obtained by Bostroem[3] who cultivated in rare instances a branching filamentous organism from bovine and human actinomycosis. This organism grew well aërobically and formed dense nodular masses or membranes of a reddish color. Bostroem did not find characteristic club-shaped forms in his colonies and was unable to produce lesions in animals like those of the disease. Results similar to those of Bostroem were obtained by Gasperini[4] from bovine actinomycosis. Wolff and Israel,[5] however, after Bostroem's work, but before that of Gasperini, studied with great care two cases of human actinomycosis and succeeded in cultivating an organism somewhat different from that of Bostroem. It grew slowly, only at 37° C., and only as an anaërobe. The colonies on agar were hard elevated nodules which gave off tooth-like extensions projecting into the agar. Microscopically the cultures showed networks of long filaments with true branching. Wolff and Israel produced tumor-like masses in animals by intraperitoneal inoculation, and in the inside of these tumors they found typical actinomyces colonies with the characteristic club formation.

More recently the subject of actinomycosis in cattle and man was investigated with great care by Wright.[6] From 13 cases of the disease in man and 2 in cattle Wright isolated organisms corresponding closely to those of Wolff and Israel. He found no difference between bovine and human actinomycosis and isolated the same anaërobic organism from both types of the disease. The present description is taken largely from the work of Wright.

**Morphology.**—In animal material the organism appears as a network of long threads which show true dichotomous branching. The terminal filaments of this network are arranged in clusters to which the term "granules" or "Drusen" is often applied and are enlarged or thickened so as to present the appearance of clubs. The nature of these clubs is still under discussion but it is held by Wright that they are degeneration products.

Agar colonies when young consist of branching filaments with radial arrangement. The branching is the true dichotomous type. Older colonies show more abundant branching, the various threads tending to break up into segments which appear as short filaments and bacillary forms. In broth the colonies when broken up show longer or shorter rods and filaments which are sometimes branched. These rod-like forms according to Wright are about the diameter of the diphtheria bacillus.

**Staining Reactions.**—The organism stains well by Gram's method and usually retains the dye. In broth cultures the organism stains irregularly, only certain parts of the cytoplasm taking the stain. One culture reported by Wright was aberrant, being Gram-negative or only faintly positive.

Not acid-fast.

**Methods of Cultivation.**—Pure cultures are always obtained with considerable difficulty and great care must be exercised. The best method according to Wright is to remove the granules from closed lesions under aseptic precaution, wash them in sterile water or broth and then crush them between glass slides. This disintegrated material is then transferred to tubes of melted 1 per cent. dextrose agar filled to a depth of 7 to 8 cm. and cooled to about 40° C. This is incubated at 37° C. and in a certain proportion of cultures characteristic colonies develop in the depths of the agar, particularly in a zone 5 to 12 mm. below the surface. From these agar cultures small bits of agar containing characteristic colonies are cut out under aseptic precautions, washed again in sterile water or broth and finally planted deep in a tube of melted 1 per cent. glucose agar cooled to 40° C. This colony if viable will now develop into good-sized masses from which transfers can easily be made. In other instances the organism can be obtained by washing the granules from the lesions and placing them on the sides of sterile test-tubes plugged with cotton and kept in the dark. After drying two to three weeks the contaminating organisms if present have died out while the actinomyces

is still viable. These viable granules transferred to melted 1 per cent. glucose agar kept at 40° C. give good growths from which subcultures may be made.

**Cultural Characters.**—*Deep 1 per cent. glucose agar* colonies develop in two to four days in the depths of the agar and are especially abundant in a zone of 2 to 4 mm. wide about 5 to 10 mm. below the surface. Above this zone the agar is free from colonies and the zone often appears as a dense grayish cloud. While the organism is thus essentially an anaërobe its growth in this zone is explained by Wright as due to the stimulating action of the oxygen absorbed from the surface. In the more recent nomenclature it would be classed as a partial tension organism or a micro-aërophil. The colonies appear as irregular, spherical or nodular, opaque, grayish-white masses attaining a diameter of 2 to 3 mm. after keeping the incubator at 37° C. a week to ten days. Microscopically the smaller colonies are composed of spherical masses of branching filaments arranged in radiations. The larger colonies consist of closely packed opaque masses without visible branching.

*Dextrose Agar Stab.*—Growth only in the depths along the line of inoculation as a dense grayish streak or line of small nodules, not extending into the medium.

*Broth.*—No surface growth. Solid whitish masses at the bottom of the tube, in the form of nodular irregular spherical structures, often forming coherent mulberry-like colonies. After long laboratory cultivation the growth in broth appears as a flaky friable amorphous mass which later becomes stringy and viscid. The broth usually remains clear, but may very rarely be clouded. The growth in broth takes place in the bottom of the tubes when kept under both aërobic and anaërobic conditions.

Surface growths on plain agar, glycerin agar, dextrose agar and Loeffler's blood-serum are usually scanty. With heavy inoculations thin patches of small grayish nodular masses may appear or a few separate colonies attaining a diameter of a few millimeters in a week or ten days. These colonies may appear as heaped-up whitish opaque irregular or rosette-shaped masses or circular masses with central elevated portions.

*Potato.*—Growth very scanty and indefinite. Occasionally a growth in the water at the bottom of the tube. No growth in potato infusion.

*Peptone.*—No definite growth.

*Litmus Milk.*—No definite growth.

No growth in beer-wort, oat infusion or on egg cultures.

Growth only at 37° C.

**Vitality.**—Culture material from broth or agar kept on the sides of sterile test-tubes at room temperature in the dark remained viable for long periods of time. In some instances transfers grew after fifty days and in one instance after eighty days. The organisms survive some days (six to ten) in broth or sugar agar and occasional strains keep alive in sugar agar for long periods at room temperature (*e. g.*, eighty-three days in one instance).

*Thermal Death-point.*—Killed by heating suspensions on a water-bath kept at 62° to 64° C. in ten to eleven minutes.

*Club Formation.*—In ordinary media and in broth containing dextrose, salt, pus, serous fluids and human, horse, and beef blood-serum, clubs were not usually formed. Occasionally positive results were obtained in broth containing blood-serum rich in fibrin (obtained through venesection in pneumonia) or pleuritic fluids yielding a fibrinous clot. The clubs when formed were found only in the filaments in immediate contact with the fluid. Club formation consisted essentially in the appearance of a hyaline eosin-staining layer about the filaments, often several times as thick. They were best demonstrated by embedding the vegetations in paraffin and staining by Mallory's method.

**Pathogenic Action.**—Cultures of actinomyces were usually without definite ability to produce progressive actinomycosis. With all cultures lesions followed inoculation which were histologically the same as the lesions in the natural disease. They appeared in the form of nodules from 1 to 12 mm. in diameter consisting of connective tissue surrounding small pus cavities. In these cavities characteristic colonies with clubs appeared. Wright obtained no evidence that the organisms had multiplied after introduction into the body. This failure to produce the disease experimentally from cultures is in line with inoculation experiments with granules taken from lesions which are usually without result.

1. Bollinger: Centralbl. f. d. med. Wissensch., 1877, xv, 481.
2. Harz: Jahresb. d. Münch. Central-Thierarzneischule, 1877–78, pp. 125–140.
3. Boström: Beitr. z. path. Anat. v. z. allg. Path., 1890, ix, 1.
4. Gasperini: Proc.-verb. Soc. Tosc. di Sci. Nat., March 4, 1894, July 7, 1895, ix, 64; 292.
5. Wolff and Israel: Virchow's Archiv., 1891, cxxvi, 11.
6. Wright: J. Med. Research, 1904–05, xiii (n. s. viii), 349–404.

### Actinomyces canis Levy

An *anaërobic* actinomyces obtained in Long's laboratory by Lange and Manasse from a suppurative phlegmon of the neck in dogs. Described by

Levy. Showed great similarity to the anaërobic organisms of Israel and Wolff. Threads more abundant on ordinary media and definite branching. Disease (actinomycosis) reproduced by inoculation into peritoneum and into testicles of dogs.

Musgrave and Clegg studied subcultures of this organism obtained from Levy and found it acid-fast and pathogenic for monkeys and guinea-pigs, producing miliary tubercle-like nodules in the peritoneum. Culturally almost identical with *Actinomyces capræ.*

Levy: Centralbl. f. Bakteriol. [etc.], 1899, xxvi, 1.

### Streptothrix interproximalis Fennel

A micro-aërophil isolated from the mouth by Fennel[1] on Martin's agar[2] and pleuritic fluid by Wherry and Oliver's system of partial oxygen tension with a culture of *Bacillus subtilis.*[3]

**Morphology.**—Highly pleomorphic, growing in plaque-like colonies of intertwined and matted threads. Five-day cultures show heavy long intertwined threads, 2 to 4 $\mu$ in width, 5 to 80 $\mu$ in length. They vary in diameter, are straight and curved, branched, and unbranched. They show distal enlargements, segmental indentations and knob-like ends. The threads may end in two equal branches, may show one or many lateral branches of equal size but with a marked constriction at point of origin. In cultures two weeks old at partial oxygen tension fantastic involution forms occur, knobs, commas, heavy crutch-like forms, horns, scrolls, and plain or spotted rods. Non-motile. No evidence of spores.

**Staining Reactions.**—Stained well by aqueous safranin, Loeffler's methylene-blue and gentian-violet. The majority of the threads are Gram-positive but some are Gram-negative. Many stain irregularly being Gram-positive at one end and Gram-negative at the other.

**Cultural Characters.**—No anaërobic growth. Slight aërobic growth. Slow growth at partial tension. Grows best at 35° C., not at room temperature. No pigment production. No odors. Best isolated on serum agar, rarely on dextrose agar.

Colonies very tenacious. Subcultures grow well on dextrose or maltose agar.

*Dextrose agar slants* show a faint change on the surface in two days, pin-point white translucent hemispherical colonies with smooth edges appearing in three days. Pinched in size in four to five days, not confluent. After six days each colony shows a nipple-like elevation in the center which has a rounded apex. Colonies remain pearly white, smooth and glistening. Largest colonies 1.5 to 2 mm. in diameter.

*Dextrose agar stab* gives only a faint growth in upper 5 mm. of the stab.

*Plain Agar Slant.*—Growth not so profuse as on plain agar and colonies very minute.

*Maltose Agar.*—Growth like that on plain agar.

*Loeffler's Blood-serum.*—Growth without liquefaction.

*Blood Agar.*—Typical growth. No hemolysis.

No growth on gelatin or potato.

Acidity in dextrose, saccharose, maltose, and galactose, none in lactose.

Differs from Goodby's *Streptothrix buccalis* in important features and therefore regarded by authors as new species.

1. Fennel: J. Infect. Dis., 1918, xxii, 567.
2. Martin: J. Path. and Bacteriol., 1911, xv, 76.
3. Wherry and Oliver: J. Infect. Dis., 1917, xx, 28.

### Mayer's actinomyces

Found in a foul empyema by Meyer. Obligate anaërobe growing only on media containing glucose and ascitic fluid. Morphologically a typical actinomyces.

Mayer: Prag. med. Wchnschr., 1887, No. 20.

### Neschezadimenko's actinomyces

Described as a streptothrix by Neschezadimenko from the pus from a chronic abscess in the right hypochondrium.

**Morphology.**—Long tangled branching threads about 0.75 to 1 $\mu$ thick and 5 to 60 $\mu$ long. Stained well by Gram, often irregularly, so that the threads appear like streptococci. Such threads 1.5 $\mu$ thick. No clubs or rays (Drusen). Occasionally terminal swelling like the diphtheria bacillus. Branching dichotamous. Stains well by ordinary anilin dyes. Decolorized by Ziehl-Neelsen's method and Rahe's method, *i. e.*, not acid-fast.

**Cultures** obtained by washing the flocculent particles from the pus in sterile water and planting on the usual media. Growth only under completely *anaërobic* conditions at 36° to 37° C. and on a few media. In *broth* with egg-yolk an abundant development after eight to ten days. The growth consisted of long threads and appeared as white granules on the walls of the tube and in the sediment on the bottom.

*Agar* colonies irregular, with projections extending from the centers of the colonies out into the media, at first gray-white, later yellow, especially in the centers. Coagulated *serum* colonies like those on agar.

No growth on potato or gelatin.

No pathogenic action demonstrated.

Neschezadimenko: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1918, xlvi, 573.

### PLAUT'S ANAËROBIC ACTINOMYCES

Plaut[1] has cultivated obligate anaërobic actinomyces (streptothrices) from three infections. In one, an abscess about the lower jaw and parotid gland, the pus was thin and fluid and contained small barely perceptible granules about the size of grains of sand. Under a low power lens these showed dark centers surrounded by lighter zones. With a high power, fine tangled interlaced threads with slightly thickened ends could be made out at the periphery. Many thin bacilli were also present but no actinomyces "Drusen."

When stained by Gram the threads were segmented with Gram-positive and Gram-negative elements. Not acid-fast. Cultures were made on a variety of media but growth was successful only in Tarozzi broth under anaërobic conditions. After twelve days at 30° to 33° C. a turbidity developed, not diffuse but containing many very minute granules which combined in large crumbly masses between the fragments of liver. Microscopically this growth revealed long mycelial threads thicker than those in the pus, tangled and entwined, with slight terminal swellings. Rarely true branching. When stained by Gram the threads were fragmented and broken up into individual thin rods consisting of a Gram-negative middle piece and 2 Gram-positive poles. The second generation in Tarozzi broth showed after eight days a yellowish-white sediment which rendered the broth turbid on shaking. This consisted of a loose mass in which individual granules were embedded. These were the size of a pin-head, hard smooth and coherent, like those observed originally in the pus. Microscopically they were made up of strongly Gram-positive diphtheroid rods with terminal swellings. In old cultures only a Gram-negative mycelium with Gram-positive granules could be made out. Eventually these showed plump terminal club-shaped thickenings like those described by Wolff and Israel in old actinomyces and which can be likened to the "Drusen" in the tissues.

Shake cultures in 1 per cent. sugar liver agar (Axenfeld's method[2]) showed after six days the granules in the depths surrounded by a zone of white punctiform colonies $\frac{1}{2}$ cm. below the surface. These later developed into delicate angular bodies with finely branched edges, the size of millet seeds. Microscopically these colonies consisted of long fine interlacing tangled threads with occasionally true branching, slender rods and oval spore-like bodies. Old cultures showed only Gram-positive rods and cocci, difficult to tell from diphtheroids.

The second case was an abscess of the lower jaw with yellow pus containing clumps of granules but no "Drusen." Microscopically this material showed Gram-positive rods, long mycelial threads and cocci. Growth was obtained in Tarozzi broth, agar shake, and agar stab cultures and in glucose liver agar. The colonies in agar were compact and under a low power showed threads with true branching. At the point of the true branching refractile granules could be detected, like those described by Neukirch.[3] Non-pathogenic.

The third case was a pleuritic exudate. The pus had the odor of butyric acid and was yellow-brown in color from broken-down blood pigment. This pus contained many granules the size of millet seeds, golden-yellow to black in color. Microscopically it revealed streptothrix threads arranged radially, thin Gram-negative bacilli and crystals of hematin, but no "Drusen." Anaërobic growth in Tarozzi broth but no growth in agar. Non-pathogenic.

These actinomyces (streptothrices) are regarded by Plaut as similar to the obligate anaërobes described by Silberschmidt, Axenfeld,[2] Wissman,[4] Neschezadimenko, and Neukirch.[3]

1. Plaut: Centralb. f. Bakteriol. [etc.], 1920, cxxxiv, 440.
2. Axenfeld: Bakteriologie in der Augenheilkunde, 1907.
3. Neukirch: Diss. Strassburg, 1902.
4. Wissman: Monatsbl. f. Augenheilk., 1913.

### ACTINOMYCES HOMINIS Bostroem

Branching thread-like organisms have been cultivated from human lesions by aërobic methods by a number of observers. They are usually non-pathogenic and are to be clearly differentiated from *Actinomyces bovis* Harz, which is an obligate anaërobe and apparently the sole cause of true actinomycosis of animals and man. As the type of these aërobic actinomyces from human lesions we may take the organism obtained by Bostroem and called *Actinomyces hominis*. It is apparently the same as *Streptothrix Aktinomyces* of Rossi-Doria and *Nocardia actinomyces* of de Toni and Trevisan. The organism grows on all the usual media at room temperature. For successful results the material must be rubbed up between glass plates or ground up in a mortar and a large number of cultures made.

**Morphology.**—In cultures the organism appears as threads 0.3 to 0.5 $\mu$ thick, thicker when stained with gentian-violet and Gram than with methylene-blue. In the depths of old blood-serum and gelatin cultures club-shaped thickenings appear at the ends of the threads. These have been explained as a gelatinization of the thread membranes. The unchanged threads appear inside them. They are 6 to 8 $\mu$ in width, very refractile and arranged in peripherally radiating strands like the "Drusen" in the tissues. When double staining with carmin and Gram is carried out the threads appear bluish-black, the clubs red.

**Cultural Characters.**—Young colonies on agar or glycerin agar are minute gray points which microscopically consist of transparent threads arranged in ray-like networks. After several days these points develop into opaque colonies which are provided on the periphery with delicate radiating processes. No pigmentation as a rule but the agar may turn brown (Rossi-Doria). An aërial mycelium formed occasionally.

*Blood-serum.*—Isolated colonies are yellowish-red, brick-red, or rosy-red and covered with a white down which consists of radiating threads (aërial mycelium). The colonies cling to the medium and when films are made only spores and broken threads are found. The true structure can only be obtained by hardening the colonies "in toto" and sectioning them. After several weeks the isolated colonies coalesce to a thick wrinkled membrane and gradually sink into the serum which is slightly liquefied.

*Gelatin Stab.*—Arborescent growth along the line of inoculation. No pigmentation or aërial mycelium. Gelatin liquefied.

*Broth.*—Large granules which break up on violent shaking. No turbidity. Rarely a few floccules or a membrane develops on the surface, made up of aërial threads.

*Potato.*—Slow growth as thick bands or skin-like knobs or granules. They are yellowish-red in color and covered by a whitish down of aërial threads.

*Milk.*—Granular masses of growth with peptonization.

*Thermal Death-point.*—Spores destroyed at 75° C. in five minutes (Domec).

Usually non-pathogenic.

Bostroem: Beitr. z. path. Anat. u. z. allg. Path., 1890, ix, 1.

### ACTINOMYCES HOFFMANNI Gruber

Described by Gruber as *Micromyces Hoffmanni* and studied by G. von Hoffmann-Wellenhof in Gruber's institute.

Branching threads which quickly break up into segments, star-shaped, or antler-shaped. Spores not observed. Growth best at 37° C. on sugar media with production of acetic acid. Injected subcutaneously in rabbits produces purulent fibrinous inflammation of the connective tissue with abscess formation.

Gruber: *Micromyces Hoffmanni* Demonstration auf. Lond. internat. Kongress, 1891. Ref. München. med. Wchnschr., 1891.

### ACTINOMYCES LIQUEFACIENS (Garten)

Reported as *Cladothrix liquefaciens* by Garten from a case resembling actinomycosis but with masses of mycelium on the sides of the abscess cavity instead of the yellow granules of true actinomycosis. Cultivated on all the usual media, best at 37° C., also on gelatin at lower temperatures. In gelatin stabs a whitish button on the surface with delicate threads projecting from the line of growth. On agar and potato wrinkled folded membranes with whitish surface covering made up chiefly of conidia. Occasionally intraperitoneal infection of rabbits and guinea-pigs produces purulent nodules in peritoneum.

Garten: Deutsche Ztschr. f. Chir., 1895, xli, 432.

### ACTINOMYCES MADURÆ (Vincent)

This organism was described originally by Vincent[1] as *Streptothrix maduræ*. It is present in the small nodes in the disease known as Mycetoma or Madura foot. These nodes contain serous pus in which small yellowish-white grains appear resembling the grains in actinomycosis. They vary in size from a millet seed to a pin's head and are made up of innumerable closely intertwined hyphæ. Under the microscope these grains are found to consist of slender closely interwoven straight or wavy filaments. These measure 1 to 1.5 $\mu$ in diameter. The filaments are radially arranged and appear to be branched. They show irregular enlargements, about 2 $\mu$ in diameter, but no clubs. In cultures the filaments exhibit the same radial arrangement and are seldom more than 1 $\mu$ in diameter. In old cultures (two weeks) the filaments are often broken up into regular ovoid segments which are larger than the filaments and constitute the fertile hyphæ. Refractile oval spores are present with sharply defined outlines. They measure 1.5 to 2 $\mu$ in width and are arranged in pairs, chains, and irregular bunches. On transfer to broth these spores give rise to short rods with rounded ends.

The organisms stain well with the usual basic anilin dyes and retain Gram's stain. The spores stain well by Gram's method and with the usual dyes.

**Cultural Reactions.**—*Actinomyces maduræ* grows well at temperatures between 20° and 40° C. and best at 37° C. It is a strict aërobe. It grows best on the following media:

*Vegetable Infusions.*—These are made from straw or hay (15 grams to the liter) or potato (20 grams to the liter). After four days at 37° C. small grayish floccules appear adherent to the sides of the flask or falling to the bottom. After three weeks, cultivation these masses attain the size of a green pea. Some turn brown in the center. Others on the sides of the flask or on the surface become pink or red in about a month.

*Meat Infusion Broth.*—Scanty growth. Small round grayish granules in about a month, the broth remaining clear. More abundant growth after several subcultures.

*Gelatin Stab.*—Scanty white growth along the line of inoculation and on the surface. No liquefaction. More abundant growth in the following medium:

| | |
|---|---|
| Potato or hay infusion, | 100 c.c. |
| Gelatin, | 9 grams |
| Glycerin, | 4 grams |
| Glucose, | 4 grams |

Neutralize and sterilize.

*Glucose Glycerin Agar.*—Abundant growth in the form of circular smooth raised colonies, yellowish-white at first, later pink and even bright red. The colonies may become very large and umbilicated, the central depression being white and the raised margins reddish.

*Potato.*—At 37° C. in five days small whitish projections appear later assuming a mulberry-like appearance. After a month the colonies become pale pink and ultimately bright red, orange, or deep red. More intense color on acid potatoes. The colonies may be covered with fine whitish dust consisting of spores.

*Milk.*—Growth without coagulation.

No growth on *serum* or *egg media*.

**Vitality.**—The organism is very resistant to drying. Cultures dried on blotting paper are alive after nine months. On potato cultures may be alive after twenty-one months. Non-spore-bearing cultures are killed in three to five minutes at 60° C. Spores resist a temperature of 75° C. for five minutes but are killed in three minutes at 85° C.

All inoculation experiments on animals negative.

Description from Besson.[2]

1. Vincent: Ann. de l'Inst. Pasteur, 1894, viii, 129.
2. Besson: Practical Bacteriology, Microbiology and Serum Therapy. Hutchen's Translation, 1913, p. 662.

### ACTINOMYCES FARCINICA (Nocard)

Found originally by Nocard[1] in a disease of cattle in Guadeloupe known as "farcin de boeuf" and characterized by a suppurative inflammation of the superficial lymphatics and glands, lymphangitis and lymphadenitis with cold abscesses breaking through and forming sinuses. Not named by Nocard.

**Morphology.**—The organism appears in the pus as a small tangled mass of fine long bacilli, the central part forming a kind of nucleus and the periphery a myriad of fine prolongations, the majority of which are branching. The organism is about the size of swine-erysipelas (rouget de porc). The same type of organism occurs in the tubercle-like nodules in the internal organs. The morphology in cultures is just like that in tissues, masses of tangled filaments with bacillary structure to be made out only on the edges. In reality the organism is a small bacillus which elongates, segments, and divides dichotomously. Old cultures are rich in spores, especially glycerin fluid cultures. They are extremely small, difficult to stain, ovoid at the ends of the organisms just where they divide dichotomously. According to Berestneff[2] this organism is acid-fast.

Vitality and virulence retained for a long time under artificial conditions. After four months at 40° C. both vitality and virulence for guinea-pigs retained. Not destroyed at 65° C. in fifteen minutes. Destroyed at 70° C.

**Cultural Characters.**—The organism can easily be cultivated aërobically on the ordinary laboratory media at 30° to 40° C. Gelatin shows no growth at 22° C. but an abundant growth at the temperature of the thermostat. No anaërobic growth.

On *agar* the organism develops in small irregular roundish projecting opaque mammillated masses somewhat thicker on the edges, yellowish-white in color, dull and powdery. After a while these lichen-like plaques unite and fuse giving the culture the appearance of a thick coarsely pleated or folded membrane.

On *potato* rapid growth occurs with the formation of small projecting shell-like or scale-like plaques, dry pale and yellow, with edges elevated from the substratum of the medium.

*Serum Gelatin.*—Growth less rapid, like that on agar but more moist.

*Broth.*—Multiplication in form of irregular whitish masses of which the majority sink to the bottom while a few remain on the surface forming a sort of roundish lenticular pellicle grayish in color with a greenish reflection, powdery, not moistened by the medium. This appearance is especially seen in glycerin bouillon. Less abundant and less rapid development in faintly acid media. No change in reaction of neutral or alkaline media even when sugar is added.

Development in *milk* without change in reaction and without coagulation.

**Pathogenic Action.**—Virulent for guinea-pigs, sheep, and cattle. Not virulent for rabbits, dogs, horses, and asses.

Intravenous and intraperitoneal injection in guinea-pigs produces in nine to twenty days lesions which are like miliary tuberculosis. In intraperitoneal inoculation the tubercular nodules are present in the peritoneum and in the various organs, spleen, liver, kidney, etc. The organs of the thoracic cavity are not involved. With intravenous inoculation all the organs are involved. In sheep and cattle intravenous inoculation gives rise to similar lesions, like generalized tuberculosis, but the animals seldom die of the condition.

Intravenous and intraperitoneal injections in rabbits, dogs, cats, horses, and asses do not produce lesions. Subcutaneous inoculation results in abscesses which open spontaneously and cicatrize rapidly with refractory animals. With sheep and cows the abscesses disappear but new abscesses develop several weeks or months after the original have gone. With the more susceptible guinea-pigs extensive phlegmonous ulcerations are produced which in general resemble the condition in the natural disease but from which the animals seldom die.

Miliary nodules were produced in monkeys by Musgrave and Clegg[3] by these organisms after nearly twenty years artificial cultivation.

1. Nocard: Ann. de l'Inst. Pasteur, 1888, ii, 293.
2. Berestneff: Diss. Moscow, 1897.
3. Musgrave and Clegg: Philippine J. Sc., 1907, ii, 477.

### ACTINOMYCES ASTEROIDES (Eppinger)

Originally found in the pus of a cerebral abscess by Eppinger[1] and described as *Cladothrix asteroides*. Same as *Streptothrix Eppingeri* Rossi-Doria[2] and Kruse.[3] Isolated by MacCallum[4] from the peritoneal exudate of a three-year-old child dying after stricture of the esophagus. Present description taken from MacCallum's which agrees with Eppinger's.

**Morphology.**—Tangled masses of branching filaments in the original pus without evidence of septum formation. Branching well demonstrated in colonies on agar plates and in broth. This is not dichotomous but lateral, the side branches being smaller than the main trunk. The tips or ends of the main stem and of the twigs are somewhat bulbous at times but generally tapered. The protoplasm is clear in the branches but irregular in the stems which show highly refractile bodies like fat droplets, which do not however stain black with osmic acid. When taken from solid media the organism appears chiefly as short bacilli or short coccus-like bodies. These are often irregular in outline and chains of bulbous or fusiform segments may occur. On potato the peculiar chalky growth is made up of fine filaments. When stained these are seen to be provided with terminal chains of coccus-like bodies which resemble the conidia of the molds. In hanging drops of broth the organism grows out into long filaments which branch and form star-like clusters with coccus-like bodies in the center.

No motility.

**Staining Reactions.**—The organism stains well with the ordinary anilin dyes, somewhat better with methylene-blue. It retains Gram's stain. The staining is not homogeneous and clear unstained areas appear in the bacillary forms and in the segments. Stained by Neisser's method from twenty-hour blood-serum cultures the organisms show bluish-black granules. According to Aoyama and Miyamoto[5] *Actinomyces asteroides* is acid-fast.

No evidence of spores by Moeller's or Abbott's methods. The terminal coccus-like bodies seen in the potato culture stain blue by Moeller's method and not red.

**Methods of Cultivation.**—Growth always as an aërobe, never as an anaërobe.

*Agar Plates.*—Small radiating colonies develop in twenty-four hours often visible to the naked eye. They grow rapidly and are well-developed in twenty-four hours, always on or near the surface. They are round, discrete, opaque, pale yellow in color, so firm and hard as to be removed from the medium with difficulty. They often project from the surface as little conical mounds with central crater-like depressions. The growth of the filaments from the colony produces a peculiar halo about it. In older cultures the colonies are larger, 3 to 4 mm., elevated and folded, pale orange in color.

*Agar Slant.*—Lumpy elevated growth made up of discrete and confluent filamentous colonies, appearing as a wide expansion and sometimes showing small spreading colonies along the sides.

*Glucose Agar.*—Colonies visible in twenty-four hours, well-developed in forty-eight hours, larger and denser than plain agar colonies. In stab cultures growth extends along line of inoculation. No gas produced.

Growth much the same on 5 per cent. glycerin agar. On hydrocele-fluid agar the growth is extremely abundant as a thick homogeneous yellow-white film over the entire surface, elevated at various points by scattered colonies.

*Gelatin Plates.*—Round yellow-white colonies of the same type as on other media. No liquefaction.

*Blood-serum.*—Abundant growth in the form of elevated nodular yellowish-white colonies becoming more or less confluent along the line of inoculation.

*Broth.*—Thin dust-like surface pellicle in twenty-four hours. The broth remains clear and at the bottom of the tube a coherent filamentous cloud appears in which minute opaque yellowish-white balls are entangled. In glucose-free broth in old cultures the pellicle appears like a very thin waxy flake sinking to the bottom on shaking. The sediment becomes much more granular in old cultures. Growth usually profuse in glucose broth.

*Dunham's Solution.*—Characteristic growth.

*Litmus Milk.*—Yellowish granular sediment. No coagulation. Gradual production of an alkaline reaction, the milk becoming deep blue after a number of days. A thin pellicle appears on the surface and the cream is tinged orange-yellow.

*Litmus Whey.*—Decolorization with the formation of a thick pellicle and sediment. Return of color when cultures are placed at room temperature.

*Potato.*—Very characteristic growth. Grayish-red dull film on the surface in twenty-

four hours. This gradually becomes thicker and nodular. A chalky-white bloom develops on the upper drier portion of the potato and gradually covers the entire surface. In older cultures the velvety growth gives rise to a peculiar tangled mass with projecting reed-like filaments or stalks.

**Thermal Death-point.**—In emulsions from agar cultures the filamentous form of the organism is killed in five minutes at 70° C., in ten to fifteen minutes at 68° C. The coccus-like bodies in emulsions from potato cultures are killed in five minutes at 68° C. and in ten minutes at 65° C. They are thus more sensitive to heat than the filamentous forms and cannot be spores.

**Pathogenic Properties.**—*Actinomyces asteroides* is virulent for guinea-pigs and rabbits by subcutaneous, intravenous, and intraperitoneal inoculation. Subcutaneous inoculation in mice produces a local lesion, the animals dying without lesions in the organs. Dogs are susceptible to intravenous inoculation and develop lesions in the internal organs. Following intravenous inoculation of small doses rabbits die after a few days, with fairly large opaque yellowish-white abscesses in the internal organs and solid nodules in the lungs. Abscesses may also appear in both the voluntary and involuntary muscles. These abscesses show microscopically tangled masses of filaments which stain characteristically by Gram's method. In animals killed soon after inoculation the beginning abscess appears as a widely distended blood-vessel packed with leukocytes and organisms. Older abscesses show necrotic material infiltrated with leukocytes surrounded by capsules of new-formed connective tissue.

After intraperitoneal inoculation nodular masses of leukocytes, fibrin, and organisms develop on the peritoneal surfaces with a marked proliferation of new connective tissue about them. Later these nodules are found to consist of a formless necrotic central mass surrounded by or enclosed in a zone of proliferated connective tissue with abundant blood-vessels. The nodules in the kidneys of rabbits inoculated intraperitoneally contain long tortuous cylindrical bodies, sometimes branched and convoluted. The round ends of these are often covered with radiating bulbous filaments resembling these described for *Actinomyces bovis*. They apparently correspond to the "sulphur grains" present in ordinary actinomyces abscesses and represent the so-called "Drusen." These "Drusen" are found to consist of a central branched cylindrical core which is exceedingly brittle and gives the reactions of calcium carbonate, effervescing on the addition of hydrochloric acid. Attached to this central core along the sides and about the tips are numerous radiating filaments with club-like extremities. This formation is regarded by MacCallum as an integral phase in the life history of the organism.

1. Eppinger: Ziegler's Beiträge, 1890, ix, 287.
2. Rossi-Doria: Ann. d. Ist. d'ig. sper. d. Univ. di Roma, 1891, i, Fasc. 4.
3. Kruse: *In:* Die Mikroorganismen (Flügge). 3. Aufl. 1896, ii, 59.
4. MacCallum: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1902, xxxi, 529.
5. Aoyama and Miyamoto: Mitt. a. d. med. Fakult. d. K. Univ. zu Tokyo, 1900, iv, 231.

### ACTINOMYCES LATHRIDII (Petruschky)

Described originally by Petruschky as *Streptothrix lathridii* and isolated from the beetle, *Lathridius rugicollis*.

**Morphology.**—Long thin threads definitely branched. In old cultures the threads show swellings, split up into segments and form chains of conidia.

Growth rapid and abundant, better at room temperature than at 37° C.

*Gelatin.*—Chalky-white coherent dry growth. Liquefaction and slight musty odor.

*Agar.*—Chalky layer, velvet-like, dry and uneven.

*Glycerin Agar.*—Moist folded membrane. Moderate musty odor.

*Broth.*—Surface growth as small white scales with dry surface. Fringed transparent flocculi on the bottom.

*Potato.*—White velvet-like expansion. No pigmentation.

*Milk.*—Coherent growth in the cream. No pigment. No coagulation.

Petruschky: Verhandl. d. Kong. f. innere Med., 1898.

### ACTINOMYCES CAPRÆ (Silberschmidt)

Cultivated by Silberschmidt[1] from the lungs of a goat which were diagnosed as tuberculosis and which showed red organisms when stained by Gabbet's method. Regarded by Silberschmidt as a streptothrix and named *Streptothrix capræ*.

**Morphology.**—Filaments varying in length, more or less branched and tangled sometimes as rods and sometimes as short filaments without branching. The long form is found in the organs and in pus and in colonies in the depths of broth. Young cultures on agar and superficial colonies on broth show short forms. Filaments very fine. On agar and on the surface of broth after one to two days at 37° C. filaments are observed varying in length, more or less branched, made up of unequal elements 1 to 10 $\mu$ or more in length, clearly separated by unstained areas. Not all the elements divide and only a true branch appears in any single element. Some of the branching filaments are like those described on the diphtheria bacillus and the tubercle bacillus. In superficial agar cultures several days old the short bacillary forms predominate. In older broth cultures more short forms appear. Colonies on solid media, serum, and agar, to which they are adherent, show only the short unbranched forms while deep colonies on these media show filaments more or less branched, some with enlargements. The change from filaments to rods occurs in all superficial colonies. Organisms from deep colonies remain filamentous.

No true spores formed, but peculiar elongated bacillary forms which often stain at both poles. These bodies are no more refractile than the filaments, are easily decolorized, show no great resistance to high temperatures and are destroyed in one hour at 70° C. and in twenty minutes at 80° C. These bodies appear to come from filaments which are dividing. Sometimes they seem to be united by a kind of membrane or capsule which is not stained by the usual methods. In consequence one gets an appearance of spore-like bodies in chains. Non-motile.

Stains somewhat according to the age of cultures, young cultures taking the stain better. Stains rapidly with concentrated dyes, Ziehl's fuchsin and anilin violet. Retains Gram's stain. Stained with difficulty with methylene-blue and thionin. In the organs and tissues best demonstrated by Gram's method and eosin. Fuchs[2] reports *Actinomyces caprœ* as acid-fast.

**Cultivation.**—Growth at room temperature, better at 33° to 37° C. when the colonies appear in twenty-four to forty-eight hours. This is especially true of sugar broth and potato. Growth only aërobic.

**Cultural Characters.**—*Gelatin.*—No liquefaction. On plates the colonies develop more or less rapidly depending on the temperature. The centers are deep brown, thicker than the peripheries which are clear, and made up of very fine prolongations more or less branched like colonies of molds. The colonies turn white in thin exposed portions. *Gelatin stab* shows isolated flocculent colonies while on the surface a dry brownish layer forms. In liquid gelatin allowed to solidify the superficial colonies show numerous prolongations. Somewhat lower the prolongations are shorter while deep colonies remain small.

*Glycerin Agar* (4 per cent.).—In stabs only surface growth. At the end of two to three days in the thermostat a colony appears, very dry, verrucose, shriveled and brownish-white. In eight to fifteen days this colony becomes very prominent with irregular surface, clear brown and appearing as if sprinkled with a white mealy or floury covering. Deep colonies very small, spherical and without characteristic aspect. On agar slant the growth appears as a dry layer, prominent and brownish as if dusted with white.

Isolated colonies may reach the size of a pea. The appearance of the colonies is characteristic. They are elevated or projecting and at the same time adherent. Part of the colony develops as a hemisphere in the interior of the agar with very fine radial prolongations while the superficial, portion is clearly but irregularly outlined. The center of the colony is flattened or crater-like. Later it is wart-like, folded or creased, dry brownish-red and dusted or powdered with white. Some colonies seem to have their true color concealed with the whitish covering while others remain clearly brownish.

No turbidity in water of condensation but with vigorous cultures a membrane which covers the surface of the liquid and runs up along the wall of the tube. Also more regular spherical colonies, whitish at first and without folds, like very small colonies of molds.

*Serum.*—Beef or horse serum made with sugar broth and solidified at a temperature below 80° C. (Loeffler's serum).

Abundant development, colonies appearing in two days. They are elevated, brownish-white and adherent. On beef serum without broth the culture appears in four to eight days in the thermostat in the form of a very delicate dry slightly brownish layer in which one can distinguish punctiform colonies. This growth bears a superficial resemblance to a culture of the human tubercle bacillus. The water of condensation remains clear and is covered, especially in the areas adjacent to the tube, by a membrane formed of small colonies which are easily broken apart.

*Potato.*—Growth delicate and whitish in the first two days, thin, elevated and rose-brown. Isolated colonies round, dry, sometimes crateriform. Colonies remain small and slightly colored when tubes are covered with gutta-percha caps. In well developed cultures the layer of growth is not uniform and one can distinguish individual colonies without a magnifying glass. The pigmentation is more pronounced than on agar. Later the aërobic cultures appear as if dusted with white particles.

*Broth.*—Good growth in plain broth and in 2 per cent. sugar broth. At the end of twenty-

four to forty-eight hours in the thermostat growth appears. The liquid remains clear, the colonies developing on the surface as concave disks, very delicate, dry, whitish as if covered with dust or powdery. When only 2 to 3 colonies are present they may have a diameter of ½ cm. or more. Generally they lie next each other, but not entangled, and form a membrane covering the entire surface of the liquid and running up along the walls of the tube.

At the bottom of the tube a grumous deposit appears with later some of the superficial colonies which have precipitated.

The surface colonies may also be more spherical, smaller and white like colonies of molds.

*Peptone water* (2 per cent.) without meat extract shows the same type of growth but the colonies remain small.

*Infusion of Straw.*—Growth only when the medium is acid. At the end of four to five days whitish colonies, isolated or entangled, which drop to the bottom of the tube.

*Milk.*—Rose-white membrane on the surface in a few days. No coagulation.

**Pathogenic Action.**—Virulent for rabbits, guinea-pigs, and white mice. With rabbits subcutaneous injection produces abscesses. Intravenous injection is followed by the formation of tubercles in various organs. These tubercles resemble those due to the tubercle bacillus, those in the lungs showing giant cells and a rapid caseation. With white mice subcutaneous inoculation produces abscesses but intraperitoneal inoculation seems to be without effect. Guinea-pigs are most susceptible. Subcutaneous injection produces abscesses and numerous tubercles scattered through the organs. Intraperitoneal injection is followed by abscess formation in the abdominal cavity with tubercles scattered over the surfaces of all the organs and in them. Musgrave and Clegg[3] have produced miliary nodules in the peritoneum of monkeys with this organism.

1. Silberschmidt: Ann. de l'Inst. Pasteur, 1899, xiii, 841.
2. Fuchs: Centralbl. f. Bakteriol. [etc.], 1903, xxxiii, 649.
3. Musgrave and Clegg: Philippine J. Sc., 1907, ii, 477.

### Actinomyces of Sabrazès-Rivière

Isolated from a lung abscess by Sabrazès-Rivière. Similar organisms found also in brain abscesses.

**Morphology.**—Wavy delicate threads with true branching. No septation of threads. Terminal spores (conidia) oval, 1 by 1.5 μ. Growth rapid, aërobic, best at 37° C. Good on media containing fat or glycerin.

*Gelatin* liquefied.

*Agar.*—Warty colonies with yellow bases and dusty-white surfaces. *Glycerin agar* projecting brownish expansion turning black with age. Surface growth on *broth.* Flesh-colored dusty-white surface growth in *milk.* Fat saponified. Strong musty odor. Yellow pigment soluble in ether usually produced. In atmosphere of pure oxygen pigment brownish.

**Pathogenic Action.**—No action on animals except when lactic acid is mixed with fourteen-day broth cultures. These produce a condition like pseudotuberculosis.

Sabrazès and Rivière: Semaine méd., 1895.

### Actinomyces gedanensis (Scheele and Petruschky)

Obtained by Scheele and Petruschky[1] from a lung abscess and from the sputum of the patient and by Batisweiler[2] from a fatal case of pyemia with lung lesions resembling tuberculosis. Originally called *Streptothrix gedanensis* I.

**Morphology.**—Long definitely branched thin threads. In tissues closely intertwined. In cultures slightly bent. In old cultures the threads are sometimes swollen, break up into segments and form chains of spores (conidia).

Growth slow and scanty, best at 37° C.

*Gelatin.*—Chalky white velvet-like dry star-shaped or hemispherical colonies at 22° C. Slow softening of gelatin.

*Agar.*—Pure white round or star-shaped dry colonies which cling fast to the media. Strong musty odor.

*Glucose Agar.*—Scanty development.

*Serum Agar.*—White colonies turning yellow and confluent. Musty odor.

*Glycerin Agar.*—White expansion which later forms a yellow crust with a whitish zone on the edges.

*Broth.*—Superficial growth as small white scales with dry velvet-like surface. On the bottom fringed transparent flocculi.

*Glycerin Broth.*—Confluent wrinkled yellow membrane with a thread-like precipitate.

*Potato.*—Scanty development of the seeded material.

*Milk.*—Coherent growth in yellow cream. No pigment production. No coagulation.

**Pathogenic Action.**—Subcutaneous abscesses and a kind of pseudotuberculosis produced in guinea-pigs. Subcutaneous abscesses in mice and rabbits.

1. Scheele and Petruschky: Verhandl. d. Kongr. f. innere Med., 1897, p. 550.
2. Batisweiler: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1924, xci, 81.

### ACTINOMYCES CANDIDA (Petruschky)

Originally described as *Streptothrix candida* or *Gedanensis* II by Petruschky. Isolated from the sputum of a twelve-year-old girl with chronic lung disease suspected to be tuberculosis.

**Morphology.**—Thin threads definitely branched, slightly curved, or bent. In old cultures the threads show swellings, break up into segments, and produce chains of conidia.

Growth rapid and abundant at room and incubator temperatures.

*Gelatin.*—Chalky-white coherent dry expansion with liquefaction.

*Agar.*—Chalky expansion, velvet-like, dry, uneven.

*Glycerin Agar.*—Moist folded membrane. Moderate musty odor.

*Broth.*—Rapid and abundant development. Small white scales on the surface of the broth with velvet-like surface. On the bottom fringed transparent flocculi.

*Potato.*—White velvet-like expansion without pigmentation.

*Milk.*—Abundant development. Coherent growth in the cream. No pigmentation. No coagulation.

**Pathogenic action** slight.

Treatment of patient with a vaccine, "Streptotrichin," was followed by slow recovery.

Petruschky: Verhandl. d. Kongr. f. innere Méd., 1898, p. 550.

### ACTINOMYCES KRAUSEI (Petruschky)

Obtained by Krause from "actinomycosis pus." Growth on ordinary media slow and scanty, both aërobic and anaërobic, only at 37° C. On agar dew-drop colonies in two days. Later rosette forms. Yellowish. No growth on potato. Broth remains clear with no surface growth but with small clumps on the bottom. No growth on gelatin. Slight growth in milk. Microscopically the cultures consist of rods sometimes with club-shaped swellings like the diphtheria bacillus, coccus-like bodies and occasional branched threads.

Krause: München. med. Wchnschr., 1899, xlvi, 749.

### ACTINOMYCES PROTEA (Schürmayer)

Described as *Oöspora proteus* by Schürmayer from an abscess of the foot diagnosed originally as tuberculosis. The pus and bits of tissue removed aseptically gave a growth in broth with a sediment at the bottom of the clear fluid. Microscopically this looked like branched tubercle bacilli.

Growth on gelatin plates in five days. Superficial colonies like "mother of pearl," iridescent whitish-gray plaques, and isolated, round colonies. The round colonies are 2 to 3 mm. in diameter in about a week and are surrounded by a soft zone of liquefaction in which fine hair-like processes appear. Colonies are later yellow to brownish in color. On glycerin agar the colonies are rosette-shaped, cartilaginous-like, white or yellow, with finely jagged edges. Deep colonies round with jagged edges about the size of a millet seed. Glycerin broth shows turbidity in twenty-four hours at 37° C. with a thin surface growth and an abundant sediment. Old cultures have a deep red-brown color.

Microscopically old cultures show coccus-like bodies and club-shaped swellings on the ends of the threads.

**Pathogenic Action.**—Intrapleural injection in mice produces hemorrhagic pleurisy and pseudotuberculosis with general emaciation. Such animals are infectious to other animals, such as mice or guinea-pigs, which develop a condition like pseudotuberculosis.

Schürmayer: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1900, xxvii, 49.

### ACTINOMYCES SPITZI Lignières and Spitz

Described by Lignières and Spitz[1] from the pus in cases of "actinophytosis" of cattle due to this organism.[2] This pus contains granules made up of tangled branching filaments which retain Gram's stain and which often are swollen at the ends. In old specimens there is a central zone of filaments and a peripheral zone of radially arranged masses, the whole forming grains like those described by Bostroem and Wolff and Israel.

**Morphology.**—The organism is very pleomorphic. In agar and broth it appears as rods of varying length, straight or curved, and resembling the diphtheria bacillus. The ends of the rods are often thickened. Curved elements, comma forms, and S-shaped forms also appear. In old cultures long streptobacillary forms are found, flexuous and branching almost at right angles.

Stains well by ordinary dyes and retains Gram stain.

**Cultural Characters.**—*Peptone Broth.*—Growth slow appearing on the third day as white or whitish-gray grains, very delicate, adherent to walls of the tube, together with a granular flocculent deposit on the bottom. Aërobic cultures have only a feeble odor. Reaction intensely acid.

*Peptone Serum Broth.*—Growth augmented by the addition of a small quantity of horse or beef serum to the broth. The growth is more flocculent and the sediment more abundant.

*Lactose Broth.*—Same type of growth. Reaction acid.

*Glucose Broth.*—Reaction acid.

*Peptone Water* (10 per cent.).—Scanty growth.

*Milk.*—Slow coagulation beginning at the bottom of the tube and complete in about a week.

*Gelatin.*—No growth at room temperature.

*Agar.*—Slow growth under aërobic conditions. At the end of forty-eight hours delicate barely perceptible transparent colonies appear, like colonies of streptococci. In three days these colonies are larger, pin-head in size and less transparent. Later they project from the agar and small white granules or grains appear in the water of condensation. On the fifth or sixth day the colonies are characteristic, either large opaque circular with a raised hemispherical center surrounded by a flat zone, or small irregular with raised borders, annular or crateriform. Later the colonies are dry, pyramidal, whitish with triangular or quadrangular bases, the surface irregularly folded. The growth resembles that of the tubercle bacillus.

Moderate growth on Würtz agar and solidified serum which is not liquefied. Little growth on potato.

*Anaërobic growth* more abundant than aërobic. Cultures give off an odor suggesting sulphureted hydrogen or certain cheeses.

**Pathogenic Action.**—No result from intravenous inoculation. Subcutaneous inoculation of cattle and sheep produces abscesses which contain grains of branching filaments and small radially arranged rays.

This organism is somewhat like the organism of Wolff and Israel and put in the same group by the authors.

1. Lignières and Spitz: Centralb. f. Bakteriol. [etc.], 1. Abt., 1904, xxxv, 452.
2. ———: Arch. de parasitol., 1903.

### ACTINOMYCES JAPONICA (Petruschky)

Found by Aoyama and Miyamota[1] in a case of streptothricosis (actinomycosis) of the lungs. Cultivated from the pus in the lungs on glycerin agar plates. Named *Streptothrix japonica* by Petruschky.[2]

**Morphology.**—Fine branching threads sometimes spirally curved. True branching but not dichotamous. Gram-positive but staining incomplete. In old cultures breaks up into segments and forms chains of spores.

*Growth* aërobic, best at 37° C. No anaërobic growth.

*Gelatin.*—Very scanty growth.

*Glycerin Agar.*—Wrinkled membrane at first pearl-gray, later yellow in color.

*Blood-serum.*—Yellowish flat colonies.

*Broth.*—Hemispherical white scales on the surface, interlacing, flocculent sediment. Fluid clear. Color in old cultures brownish.

*Potato.*—Originally chalk-white granules which later coalesce. The color becomes gradually brownish-yellow.

*Milk.*—Yellowish membrane. No coagulation.

*Sterile Water.*—Definite superficial growth.

*Glucose Solution* (5 per cent.).—Growth like that on water.

**Pathogenic Action.**—Intraperitoneal and intrapleural injection of guinea-pigs produces hemorrhagic inflammation with formation of nodules.

1. Aoyama and Miyamota: Mitt. a. d. med. Fakult. d. K. Univ. zu Tokyo, 1900, iv, 231.
2. Petruschky: Die pathogenen Trichomyceten und Trichobakterien. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, v, 267.

## Birt and Leishman's Actinomyces

Described as an acid-fast streptothrix by Birt and Leishman[1] from the sputum and from pus from the pleural cavity of a fatal lung infection.

**Morphology.**—The organism occurred in the pus as a fairly open network of long thin threads, segmented and showing lateral branches which came off at a right angle. They were about 0.5 $\mu$ in width and showed no clubs or spores. In cultures they appeared as a fine branching network of mycelial threads, together with short coccus-like forms produced by the breaking up of the terminal threads into oval segments. These so-called "arthrospores" (the conidia of later writers) are produced abundantly on dry media while in fluid media the growth consists chiefly of tangled branching filaments.

**Staining Reactions.**—Threads in the pus stain well with all basic anilin dyes and retain Gram's stain. Treated by Ziehl-Neelsen carbolfuchsin and decolorized by 25 per cent. sulphuric acid and alcohol they remain deeply stained. In young cultures both threads and arthrospores are intensely acid-fast but later, as the cultures age, the threads lose this property and decolorize, while the spores remain acid-fast. In old cultures containing sterile synovial fluid dark almost black beads occur in the threads on staining by Weigert's method like the "arthrospores" of the tubercle bacillus demonstrated by Coppen-Jones (1895).

Non-motile. No clubs or involution forms.

**Cultivated** at 22° C., at 37° C., and at higher temperatures like the thermophilic bacteria. This growth took place on potato after forty-eight hours at 46.5° C., but not at 50° C. Not destroyed at 75° C. in five minutes but destroyed at 75° C. in twenty minutes and by momentary exposure at 100° C. Growth only aërobic. Vitality considerable, a dried agar culture nine months old being successfully transplanted.

*Gelatin Plates.*—Small white circular colonies develop at 22° C. in five to seven days. Under low power they resemble hemispherical balls of cotton wool, snow-white by reflected light and yellowish-brown by transmitted light. Maximum diameter about 1 mm. Gelatin stabs show surface growth like that on plates with a slight expansion down the needle-track.

*Agar.*—Rapid growth. After thirty-six to forty-eight hours snow-white dry powdery growth which a few days later takes on a delicate pale coral-pink tinge where the growth is thickest. This pink color appears on nearly all media exposed to the air. Water of condensation clear. A few rugged granules may appear in the agar slope, about 1 mm. in size. Growth not adherent but easily lifted from the agar.

*Nutrient Broth.*—Delicate white specks on the surface in twenty-four to thirty-six hours at 37° C. (described by Besson as water-lilies[2]). These specks increase slowly in size, become spherical, dry, and coral-pink above the surface, wooly and white below. The growth may extend up the sides of the tube but the rosettes as a rule remain separate and after one to two weeks sink to the bottom. Broth remains clear and odorless, alkaline reaction unchanged. No indol. Growth increased in broth by addition of a little sterile synovial fluid.

*Potato.*—Copious dry white growth in twenty-four to forty-eight hours giving the appearance of a splash of plaster of Paris. It never extends more than 2 to 3 mm. from the needle-track, may become granular, warty, and edged but not wrinkled. The growth becomes coral-pink on the third or fourth day.

*Milk.*—Surface growth of isolated rosettes like that in broth. No clotting. Proteins gradually digested with a deposit of rosettes and casein. Reaction alkaline.

*Blood-serum.*—No growth.

Scanty growth in sterile tap water like that in broth.

**Pathogenic Action.**—Intraperitoneal inoculation fatal to guinea-pigs in five to six weeks, large collections of caseous matter appearing between omentum and intestines. Subcutaneous injection produces similar caseous masses which break down into ulcers and then heal.

1. Birt and Leishman: J. Hyg., 1902, ii, 120.
2. Besson: Practical Bacteriology, Microbiology, and Serum Therapy. Hutchens' translation, 1913, p. 655.

### Actinomyces polychromogenes (Vallée)

Originally reported by Vallée as *Streptothrix polychromogenes* and isolated from the blood of a horse dead of acute pasteurellosis.

**Morphology.**—Long filaments uniformly segmented and beautifully branched. Occasionally slight terminal enlargement. Stains well by Gram-Nicolle's method and retains the dye. Stains also with 1 per cent. Borrel's blue and with carbol-thionin.

**Cultivated** easily on ordinary media. Aërobic.

*Broth with Peptone.*—Fragile colorless surface growth in fifteen hours. At the end of three days this becomes pale salmon-red. After eight to fifteen days the membrane breaks up and sinks to the bottom as a viscid sediment.

*Agar with Peptone.*—Salmon-red expansion in forty-eight hours. Growth increases in size and after a month becomes old-rose in color.

*Gelatin.*—Growth in forty-eight hours at 18° C. like that on agar. No liquefaction.

*Potato.*—Gray, rosy growth in sixteen hours gradually becoming yellowish-red. On placing the cultures at room temperature the color turns to vermillion.

*Milk.*—Growth like that in broth. No coagulation.

Growth at 18° to 38° C. and also at higher temperatures, 42° to 47° C., with pigment production.

Not destroyed at 60° C. in five minutes, but destroyed at 65° C. in the same time.

Pigment soluble in ether and chloroform.

Not definitely pathogenic. Rarely a progressive loss of weight in guinea-pigs possibly from toxemia.

Vallée: Ann. de l'Inst. Pasteur, 1903, xvii, 288.

### Zimmermann's actinomyces

Isolated by Zimmermann from the caseous nodules on the peritoneal surfaces of a guinea-pig previously inoculated with a myxomycete (*Plasmodiophora carassiba*). It grew in the form of white sugary colonies on agar, which later turned yellow and finally rusty. The growth on potato resembled that of the tubercle bacillus. Smears showed acid-fast branching filaments. Pathogenic for dogs, rabbits, guinea-pigs, mice, chickens, and frogs. Inoculated intravenously it killed rabbits in three to five days. Intraperitoneal injections killed guinea-pigs in four to six days. The peritoneal surface was studded with pseudotubercles which showed no giant cells. Zimmermann believed his organism was like Eppinger's *Actinomyces asteroides*, but more virulent.

Zimmermann: Pest. med-chir. Presse, 1903, xxxix, 704.

### Actinomyces valvularis Luginger

Described by Luginger as *Streptothrix valvularis destruens bovis* and derived from a fibrino-purulent endocarditis in cattle. Gram-positive. Forms threads in cultures with true branching. No growth on gelatin or potato. Produces purulent pleurisy and pseudotuberculosis in sheep and subcutaneous abscesses in goats and rabbits.

Luginger: Monatschr. f. Tierheilk., 1904.

### Actinomyces Freeri Musgrave and Clegg

Isolated from a case of the ochroid variety of mycetoma occurring in the Philippine Islands by Musgrave and Clegg and named by them *Streptothrix freeri*. Organism present in large numbers in the discharges from the open sinuses of the affected foot, and in the diseased tissues after amputation.

**Morphology.**—In fresh coverslip preparations various forms of the organism are present. The granules, the larger of which are of the "fish roe" type, are dull yellowish-white when large, whitish when small. They are of a tough dough-like consistency and show evidences of considerable elasticity. They vary in diameter from 0.25 to 5 mm. or more, and are of diverse shapes, the very small ones being usually oval. When pressed out on a cover-glass and examined under a low power they somewhat resemble *Actinomyces hominis*. The ray formation however is a much less distinctive feature and the "club" appearances are absent or much less noticeable. Under high power the smaller granules are seen to be made up of a mass of interwoven fungoid filaments with more or less definite radiation at the margins. The central portion in larger and older granules contains fewer of the filamentous forms, but there are numerous bacillary and coccus-like varieties together with crystals, bright shining small round bodies and débris, the whole making up a more or less dense mass. Outside of this mass is a zone of interlacing filaments, in which cells and débris are embedded, while the outer fringe is made up largely of terminal branches, loops and hyphæ. Occasionally definite clubs may be seen. The organism may also appear in the fresh material as a somewhat skein-like mass of filaments or mycelia, with hyphæ and occasional round forms. Other types are single filaments or small round glistening bodies lying free in the secretion, the nature of which is not clear. Under the highest powers of the microscope the hyphæ-like forms seem to be made up of a delicate membrane covering a homogeneous and unbroken core. In the older mycelial types the outer membrane appears to be irregular in thickness and density and absent in places. The inner portion is also frequently and irregularly broken and thus the appearance of bacillary and ovoid or coccal sections is produced. Branching forms are plentiful, the branches first appearing as buds, which grow to hyphæ from the side of the mycelial thread. True dichotomous division has not been observed. The small round bodies mentioned above often appear to have a double containing wall, and vary in diameter from 3 to 10 $\mu$.

**Staining Reactions.**—Stains well by ordinary anilin dyes, by the Gram-Weigert method. Both acid-fast and alcohol-fast, particularly in the older filaments. Stains bright red by the Ziehl-Neelson-Gabbet method.

**Cultivated** from the sinuses and tissues of the amputated foot without especial difficulty upon a variety of media. It grows only as an obligate aërobe and best at 37° C. upon potato, sugar-containing media and glycerin agar.

*Potato.*—Growth visible in twenty-four hours, luxuriant in seventy-two hours. Colonies at first small, discrete, and slightly raised, of a delicate pink color. Later the colonies become larger at the base and raised to a height of at least 0.1 mm. The upper portion of the colony appears as a short thread which often folds upon itself giving it the appearance of being umbilicated. The whole inoculated surface finally changes to a heaped-up mass which has a yellow-ochre color in the center, with a pinkish or white periphery. The medium becomes darkened and moist. The growth may be lifted in heaps on a platinum loop. When placed in water small flat particles, from delicate pink to yellowish-brown in color, resembling dry bran, float upon the surface. On shaking the vessel, the particles adhere to the sides.

*Glycerin Agar.*—Growth appears after three days, later heaped-up, the medium remaining moist. Colonies at first discrete, but they soon become confluent but not diffuse; they are raised and in some instances have the umbilicated appearance observed on potato. Older cultures resemble the tubercle bacillus. The central portions are yellowish, the periphery pink to pinkish-white. Growth readily lifted.

*Agar*, 1 per cent. acid and 1 per cent. alkaline to phenolphthalein. After four days growth appears as a smooth white and glistening, very slightly raised mass. The colonies do not develop the central pit seen on potato, glycerin agar, and sugar-containing media, produce no yellow pigment, but assume a porcelain-white, later a delicate diffuse pink color.

*Loeffler's Blood-serum.*—Growth slower than on other media. Pigmentation and growth very similar to those on plain agar.

*Glucose Agar Stab.*—No growth along the line of inoculation. Luxuriant surface growth forming a heaped-up center with a wrinkled periphery. Center yellow, periphery pink to pinkish-white.

*Gelatin.*—Growth like that on glucose agar. No liquefaction.

*Lactose-litmus Agar Stab.*—Growth like that on glucose agar. No change in reaction.

*Broth*, alkaline and acid. Floating flat particles appear on the surface after three days. On shaking, these particles adhere to the sides of the tube. Later a sediment in the form of a tenacious mass settles to the bottom. The medium does not become cloudy, but on long incubation it changes to a darker color.

*Alkaline Litmus Milk.*—Growth appears after three days on the surface in the form of dry flat particles which later become confluent, forming a heaped-up yellowish mass. A tenacious sediment forms at the bottom of the tube; in this portion of the medium the milk is gradually decolorized, but does not become red. No coagulation.

*Potato Infusion.*—Growth like that in broth. No pigment. No change in the medium.

Growth at room temperature and at 37° C., but much slower and with less pigment at the lower temperature.

No growth in cultures after exposure to 70° C. for fifteen minutes.

**Pathogenic Action.**—Pure cultures produce the disease in monkeys, guinea-pigs, and dogs by intraperitoneal inoculation. Subcutaneous inoculation of material into rabbits, guinea-pigs, dogs, and monkeys produces small nodules at the site of inoculation which soon undergo resolution and heal. Pure cultures of the organism obtained from the discharges. Intraperitoneal inoculation of rabbits and pigeons and intravenous inoculation of rabbits negative. Direct injection of fresh material into the foot of a monkey after incision and scraping the metatarsal bones, produces suppurative lesions with necrosis, the organism being cultivated from the discharge. Similar lesions produced by direct injection into the ball of the foot.

Musgrave and Clegg: Philippine J. Sc., 1907, ii, 477.

### Actinomyces luteola (Foulerton)

Reported by Foulerton as *Streptothrix luteola* from a lung infection, from a case of conjunctivitis and from an abscess connected with a carious tooth.

Other organisms in this group described by Foulerton are:

*Streptothrix hominis* I, a slow-growing organism like the tubercle bacillus from 2 cases of pulmonary streptothricosis, from two abscesses in connection with the jaw, from a case of primary infection of the tonsil with extension to the tissues in the front of the neck and from an abscess in the front of the cheek.

*Streptothrix hominis* II.—This resembles Species I but grows more freely and differs in its pathogenicity for lower animals. It was isolated from an abscess of tongue.

*Streptothrix hominis* III.—Obtained from a case of renal streptothricosis.

*Streptothrix hominis* IV.—Obtained from 2 cases of appendicitis and one right iliac abscess.

Foulerton: Lancet, 1905, i, 1200. *Ibid.*, 1906, i, 970.

### Actinomyces variabilis Cohn

Cultivated by Cohn from the pus in the bladder in a case of cystitis and from the prostate.

**Morphology**—Branching threads of various length in the pus, with terminal swellings. No true actinomyces granules in the pus. In cultures the organism appears as a branching mycelium which segments into round or oval elements. From the colonies the organism usually appears as short thick rods in a mass of branched threads. No spores.

**Staining Reactions.**—Stains well with anilin dyes, best by Gram's method. Not acid-fast Stained by Neisser's stain the long threads are filled with granules stained blue. Some of the long threads are uniformly blue-black, others contain individual ovoid blue-black granules. The coccoid elements do not take Moeller's spore stain.

**Cultivated** at 37° C., at 42° C., and at 45° C. Destroyed at 60° C. in one hour. Growth also at room temperature. Vitality retained in broth for five months and in milk fifteen months. No odor in the cultures. Aërobic growth only.

*Agar Plates.*—Small colorless or pale green round colonies which gradually become button-like. Under low power these colonies appear as a tangled mass of threads. The largest colonies are round, with smooth edges, pale brown, finely granular, not larger than a poppy seed As the colonies get older they become more button-like, surface folds appear until the center is star-shaped and the edges raised. They are firmly adherent to the medium.

*Agar Slant.*—Similar isolated colonies or a surface growth as a uniform smooth velvety colorless expansion with pale brown roundish masses appearing in it after several days. Water of condensation clear with occasional small grayish-white flocculi.

*Agar Stab.*—Growth chiefly on the surface as a round pale brown disk with jagged edges and gray surface. Gradually a brown granulation appears in the upper part of the line of inoculation.

Agar colonies are sometimes orange-yellow. The surface of old agar stabs forms a conical projection, is deeply folded and is sparsely covered with a white dust.

*Gelatin* growth like that on agar, turning orange-yellow in four to five days. No liquefaction.

*Broth.*—Colorless flat roundish membrane about 1 to 2 cm. in diameter in one to two days. On slight shaking this sinks to the bottom of the tube, and growth continues as a cloudy colorless mass. Fluid remains clear.

*Potato.*—Colorless expansion in two to four days turning yellow in two weeks, then orange-red and in six weeks ochre-brown. Surface granular.

No growth in *water* or *physiological salt solution*. Scanty growth in *peptone*.

Small white colonies on *endo-agar*, turning rose-colored in eleven days. Bright orange yellow membrane on *milk*. No coagulation In *sugar media* the organism grows abundantly and produces its orange-yellow pigment early. No *gaseous fermentation*. No *hemolysis* on blood agar.

**Pathogenic** to guinea-pigs, producing on subcutaneous inoculation abscesses which contain yellow caseous masses. Intraperitoneal injection also fatal and leads to formation of small grayish-white nodules in the internal organs. In rabbits subcutaneous injection leads to local changes only, without generalized infection. In rabbits both intraperitoneal and intravenous injection is followed by general and fatal infection. The pathological picture is like that descrbed as pseudotuberculosis. Small yellowish-white nodules, poppy seed or sago kernel in size, are present in the various organs chiefly in the kidneys, liver, spleen, and diaphragm, but also in the heart, lung, and intestinal wall. In early stages these nodules are soft, easily broken up, viscid and contain leukocytes, round cells, detritus and threads of bacteria. Older nodules are hard, broken with difficulty and consist of a yellowish-white viscid mass inside a hard grayish-yellow wall. The viscid mass contains leukocytes, round cells, epithelioid cells and actinomyces threads, short thick and frequently branched. Microscopically the young nodules show a central zone made up of round cells and epithelioid cells and an outer zone of connective tissue threads with spindle-formed nuclei and granular leukocytes. Later giant cells are present in these nodules and a transformation occurs in the actinomyces threads which become thicker, irregularly spindle-formed and frequently branched. Typical actinomyces "Drusen" are thus formed, consisting of clubs, mycelium and connective-tissue cells.

This organism is apparently the only aërobic actinomyces which produces "Drusen" on subcutaneous inoculation.

Cohn: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1913, lxx, 290.

### STREPTOTHRIX TARAXERI CEPAPI Schottmüller

Isolated by Schottmüller from a case resembling rat-bite fever following the bite of a South African squirrel. Found in the pus of the skin lesions and in the blood of the patient. The pus when stained by Pappenheim, Giemsa, or Gram showed delicate sharply stained straight or curved rods, the length of the tubercle bacillus. Some three or four times as long and not showing segmentation. Gram-negative. Some forms long and thread-like. Organisms occasionally granular. No branching. Only slight growth on artificial media, chiefly in broth.

Pathogenic to apes by intravenous inoculation.

Schottmüller: Dermat. Wchnschr., 1914, lviii, Ergänzungsheft, 77.

### ACTINOMYCES MURIS RATTI Schottmüller

Originally obtained by Schottmüller[1] from a case of rat-bite fever.

The same or a closely similar organism was isolated by Blake[2] from the blood during life of a fatal case of rat-bite fever and from the blood at autopsy.

Blake's description follows:

**Morphology.**—Slender filamentous branching organism growing in interwoven masses and not manifesting a definite radial arrangement at periphery of the colonies. Young cultures show filaments varying greatly in length, curved, wavy, or straight, many showing true branching and staining homogeneously. After eighteen to twenty-four hours' incubation the filaments fragment and branching forms rapidly disappear. Filaments now appear as chains of bacilli varying in length. They no longer stain homogeneously but show numerous granular or beaded forms. The filaments may be spindle-shaped, ball-shaped, oval, or show terminal club-shaped swellings and chains of coccus-like forms. Old cultures show rods varying in length, straight or slightly curved, beaded, resembling the pleomorphic

bacilli. Non-motile. No true endospores present. No capsules. Club-shaped forms do not show the characteristic sheath of actinomyces as described by Wright in *Actinomyces bovis*.

**Staining Reactions.**—Stains readily with methylene-blue, gentian-violet, carbolfuchsin, pyronin, and Bismarck brown. Gram-negative. Neither acid- nor alcohol-fast.

*Grows* aërobically and less abundantly as a facultative anaërobe.

*Killed* by exposure to 60° C. for ten minutes. Cultures die out at 37° C. in five to eight days.

On ice cultures remain viable seven to fourteen days.

Optimum temperature 37° C. Little growth at room temperature.

Optimum reaction for media between neutral point and 2.0+. No growth in media of greater alkalinity than 1.5— or greater acidity than 3.0+. No pigment.

**Cultural Characters.**—*Gelatin.*—No growth.

*Loeffler's Blood-serum.*—After twenty-four hours at 37° C. moderately abundant growth of discrete, whitish, pin-point, circular, sharply marginated, elevated, smooth, glistening, moist, non-mucoid colonies. Whitish flocculent growth in water of condensation. No liquefaction. No increase in size of colonies after twenty-four hours but they tend to coalesce if closely aggregated.

*Human Blood-serum.*—Very scanty growth in twenty-four hours at 37° C., like that on Loeffler's serum.

*Ascitic Agar.*—No growth of organism when first isolated. After subculturing on Loeffler's blood-serum two and one-half months, growth was obtained on ascitic agar like that on blood-serum.

*Ascitic Broth.*—(Plain broth 4 parts, ascitic fluid 1 part.) After eighteen to twenty-four hours at 37° C. a whitish flocculent growth appears at the bottom of the tube and to some extent along the sides. Medium clear. No surface pellicle. Growth ceases after twenty-four to thirty hours.

No *growth* in *plain* and *dextrose agar*, *plain* and *dextrose broth*, *ox-bile*, *litmus serum water*, and *litmus milk*.

**Fermentations.**—No growth in litmus serum water containing levulose, lactose, dextrose, mannite, maltose, saccharose, or dextrin. Good growth in inulin, lactose, mannite, raffinose, saccharose, and salicin broth to which ascitic fluid is added (1–4) but no change in reaction after seven days.

**Pathogenic Action.**—Very slightly pathogenic for rabbits and white rats, producing a local inflammatory and proliferative reaction at the site of subcutaneous inoculation with general lymph-node hyperplasia. Not pathogenic for guinea-pigs.

An organism closely similar to Schottmüller's and to Blake's was observed in smears and isolated in pure culture from the lungs of rats with bronchopneumonia by Tunnicliff.[3] This organism occasionally produced acute lesions in the lungs of rats inoculated intraperitoneally with material from ascitic broth and blood agar slants. In view of this observation and the recent work on rat-bite fever in which spirochætes have been shown to be the etiological agent of the disease, it seems probable that Schottmüller's case and Blake's case may be regarded as infections of streptothrices (actinomyces) arising as a result of rat-bite.

1. Schottmüller: Dermat. Wchnschr., 1914, lxviii, Ergänzungsheft, 77.
2. Blake: J. Exper. M., 1916, xxiii, 39.
3. Tunnicliff: J. Infect. Dis., 1916, xix, 767.

### Bloomfield and Bayne-Jones' Actinomyces

Described as a streptothrix and isolated by Bloomfield and Bayne-Jones from a liver abscess with a thick greenish foul-smelling pus probably secondary to a low grade pulmonary streptothricosis.

**Morphology.**—Interlacing mycelial threads, not very highly refractile, non-septate and single, contoured in hanging drops. Threads about 0.5 $\mu$ in diameter and dotted with highly refractile round bodies (spores ?), especially toward the periphery. True branching. No motility. No radial arrangement of threads and no clubs.

**Stains** well with carbol-gentian-violet, methylene-blue, Bismarck brown and carbolfuchsin. Gram-negative. Not acid-fast.

**Cultivated** originally on ascites dextrose agar where the colonies appeared in a sharply

demarcated zone about 2 cm. below the surface. The largest colonies reached a size of 1 mm. in diameter, gray, opaque with a slightly darker center and slightly irregular edges.

Subcultured aërobically and anaërobically on ascites dextrose agar, dextrose agar, plain agar, glycerin agar, blood agar, Loeffler's serum, potato, gelatin, and in plain and dextrose broth and milk. No growth under completely aërobic or anaërobic conditions. Growth apparently only under partial oxygen pressure, as in deep tubes of ascites dextrose agar. Most abundant growth in ascites dextrose broth, partial pressure being secured by varying amounts of pyrogallic acid and alkali. Here the colonies grow along the walls of the tube, subsequently dropping to the bottom. All cultures have a peculiar, foul odor like that of the original pus. Growth only at 37° C.

**Pathogenic powers** low. Subcutaneous inoculation in guinea-pigs produces necrotic areas, with greenish seropurulent contents.

Bloomfield and Bayne-Jones: Johns Hopkins Hosp. Bull., 1915, xxvi, 230.

### Evans' actinomyces

Described as a streptothrix (Nocardia) by Evans from a series of general infections of the cow's udder. It was isolated from 18 of 21, or 85.7 per cent. of the samples studied.

**Morphology.**—Long branching mycelial threads which break up into rods of varying length. These rods in turn are converted into coccoid forms which occur isolated or in chains held together by the remnants of the mycelium. On dry media a powdery growth occurs in which true conidia are formed at the ends of lateral hyphæ.

**Staining Reactions.**—Gram-positive. Coccoid bodies and conidia acid-fast when stained by carbolfuchsin and decolorized with 2 per cent. hydrochloric acid in 95 per cent. alcohol. Also acid-fast when decolorized with 5 per cent. nitric acid. Not acid-fast to 25 per cent. nitric acid.

**Cultural Characters.**—Discrete colonies about 1½ mm. in diameter on slant agar, wrinkled with ridges radiating from a depressed center. The growth may be confluent with a wrinkled surface. It is tough and adherent, cream-buff in color. The agar is slightly browned in old cultures. When the agar is thin and dry there is often a white powdery growth. In agar shake cultures growth takes place on the surface and on the wall of the tube for about 10 mm. above the surface. Granules, no turbidity in broth. Abundant brownish growth on potato with a honeycomb surface. No fermentation of dextrose, maltose, saccharose, lactose, starch, or mannite in broth cultures and no reduction of the hydrogen-ion concentration of the medium. No decomposition of nitrate or asparagin. No growth on gelatin at 20° C. Litmus milk rendered alkaline. Destroyed at 56° C. in thirty minutes.

Evans: J. Infect. Dis., 1918, xxiii, 373.

### Actinomyces putorii (Dick and Tunnicliff)

Reported as *Streptothrix putorii* by Dick and Tunnicliff and isolated from the blood of a patient bitten by a weasel.

**Morphology.**—Non-motile slender curved branching threads, varying a good deal in length with bacillary and coccal forms. Branching most marked in young cultures. Ball and club-shaped terminal swellings. Marked pleomorphism. *Stains* with ordinary dyes. Gram-negative. Not acid-fast.

**Cultivated** best aërobically and also as a facultative anaërobe. Growth generally occurs in twenty-four to forty-eight hours on blood agar and on Loeffler's blood-serum as discrete, colorless or grayish-white, pin-point, circular, elevated colonies with sharp margins. At first they appear dull, later become glistening. Medium not changed. Growth in dextrose blood agar is more profuse and grayish-yellow in color. The growth in ascitic broth is slight but whitish and flocculent. Growth also occurs in inulin, salicin, maltose, mannite, saccharose, raffinose, lactose, and dextrose broths to which ascitic fluid is added (1–4) but there is no change in reaction. There is slight growth only in plain and dextrose broth and milk; on plain and dextrose agar a yellowish-white glistening growth develops. Organism killed by exposure to 57° C. for half an hour.

Pathogenic action slight. Guinea-pigs and white rats died occasionally from intraperitoneal injections but the organisms were not recovered from the organs.

Dick and Tunnicliff: J. Infect. Dis., 1918, xxiii, 183.

### ACTINOMYCES CAMELI (Mason)

Isolated by Mason from a camel dying after a short illness and showing at autopsy lesions having considerable resemblance to tuberculosis. The lungs contained solid irregular masses of a grayish color up to the size of a broad bean and innumerable semitranslucent spherical nodules resembling young miliary tubercles. Yellowish-gray nodules also in the lymphatic glands, kidney, and liver. Films from various lesions showed a streptothrix which was grown in pure culture from several of the lesions. Originally named *Streptothrix cameli* by Mason.

**Morphology.**—Mycelium composed of irregularly branching hyphæ without visible sheath or segmentation. The hyphal threads are frequently of great length and about the width of the tubercle bacillus.

**Stained** homogeneously by Gram's method, but frequently showing a beaded appearance, unstained or faintly stained areas appearing between the stained portions. Many short threads also occur, bacillary in form, some beaded and some uniformly stained. The substance of the hyphæ tends to break up into minute granules and empty sheaths are seen which no longer retain Gram's stain. Many hyphæ occur with irregular and not dichotomous branching and show fusiform enlargements measuring about 1.5 $\mu$ in length by 1 $\mu$ in width, chiefly placed lengthwise in the course of a hypha. They may also be placed at the point of origin of a branch and more rarely in a terminal position, giving a somewhat drumstick appearance. These are strongly Gram-positive and according to Mason may represent arthrospores. Free spores are also numerous.

No club formation. No rosettes. No grains.

Acid-fast by Ziehl-Neelson but immediately decolorized in alcohol. Artificial cultures lose this property but regain it on animal passage. Stains well by Gram and Claudius methods. Stains faintly with aqueous solutions of methylene-blue and moderately well with Loeffler's blue, gentian-violet, and methyl violet.

Films from cultures show the characteristic streptothrix appearance as mycelia, hyphæ, beaded hyphæ, spore-formation in rows, bacillus-like forms and free spores as seen in films from the lesions and in sections. Vegetative elements and spores from cultures are Gram-positive but not acid-fast.

Spores stand desiccation for long periods. Agar cultures dried and kept in the dark are viable after eight months, when kept in diffuse light are viable after a month.

**Grows** well aërobically but not anaërobically on ordinary culture media at 37° C. and slowly at 22° to 25° C. All cultures give off a strong moldy odor.

On *agar* it grows as snow-white, rounded, flat colonies, slightly raised in the center, firmly adherent to the surface of the medium, in which they are shallowly embedded and leave a slight erosion when the colony is removed. At 37° C. growth is visible in twenty-four hours. The colonies may coalesce and form a rough nodulated snow-white membrane which is firmly adherent to the medium. The growth may remain snow-white or may turn slightly pink after fifteen days. Maximum growth in twenty-five days. Color of medium not altered.

Glucose agar growth like that on plain agar. No growth in stab cultures.

On solid blood-serum growth is visible in forty-eight hours as minute rounded white colonies, firmly adherent to the medium. At seventy-two hours the colonies are about 1 mm. in diameter, in five days 2.5 mm., with slightly darker centers, round or oval in shape. At one month the colonies are still discrete and white or faintly pink. Medium not liquefied nor altered in color. Gelatin liquefied by a growth of white spherical colonies which fall to the bottom. Bouillon shows growth at the surface as flat colonies, at the bottom as spherical colonies, or both at the top and bottom. Colonies at first snow-white. After about a week the growth tends to creep up the side of the tube. The bouillon remains clear. After fifteen days the growth may be snow-white or turn faintly pink. After a month the surface growth is much stronger than the deep colonies and the pink color more marked.

Potato growth a snow-white, slightly wrinkled pellicle with an irregular outline. It may remain snow-white or turn faintly pink after about fifteen days. Color of medium not changed. No diastatic action.

Litmus milk, growth at the surface and the bottom. After about five days red litmus will turn blue. At fifteen days the milk separates into a clear-greenish fluid above and an opaque precipitate below. At thirty days the alkalinity is about five times that of control milk.

On milk, growth appears first at the surface. On the fifth day the milk turns pink and on the next day separates into three layers of about equal depth. The top layer is translucent and brownish-pink in color. The second layer is more opaque and lighter in color, while the bottom layer is quite opaque and lightest in color. After fifteen days the fungus

is seen as a rich corrugated pink growth on the surface, the milk appearing as a clear serum with a dense precipitate at the bottom. No further change in thirty days.

**Virulent** to guinea-pigs on subcutaneous inoculation. The animals die on the seventh day and show purulent masses at the site of inoculation with the organisms uniformly distributed in the body. Subcutaneous inoculation produces abscesses in white rats. Intravenous inoculation in camels produces lesions like those seen in the natural disease.

Mason: J. Trop. M. and Therap., 1919, xxxii, 34.

### Rodella's Actinomyces

Found in an abscess about the teeth by Rodella. The pus contained granules, millet seed in size, round or lens-shaped, *i. e.*, biconvex, grayish-yellow. Microscopically the centers were a mass of threads surrounded by a zone of pus corpuscles. Threads Gram-positive and irregularly stained with a kind of sheath containing an intense violet capsule. No clubs. Right-angled branching. Ellipsoid bodies present, either conidia or clumps of volutin. Also Gram-bacilli, often curved, with round or angular ends.

**Cultivated Aërobically.**—On serum plates colonies appear in three to five days, sunken, wavy, compact, leathery, dirty-gray and lifted "in toto" from the medium. Colonies consist microscopically of thick beautifully branched threads, interlacing and coiled and round coccus-like bodies (spores ?).

Threads Gram-positive.

*Broth.*—Irregular white sediment in five to six days, fluid clear.

*Sugar Broth.*—More abundant growth. Fluid clear. Occasionally millet seed- or pea-sized spheres, compact, with white center and light viscid periphery. These spheres consist of rods and long interlacing threads.

*Gelatin.*—No growth at 22° C. Some increase at 37° C. in sugar gelatin.

*Milk.*—Homogeneous clotting on second day, not from acid but from enzymes. Clot gradually and within a week completely peptonized.

*Agar.*—Colonies develop rarely. When present they show microscopically irregular forms, wavy edges, often with projections and concentric layers. They look a little like lactic acid bacilli or anthrax colonies.

Superficial colonies round and regular. Deep egg-shaped, ellipsoid with sharp contours, coarsely granular.

After three to five days the colonies are covered with white dusty powder, easily removed with a platinum needle. The growth often forms uneven white irregular elevations like pieces of chalk.

Microscopically this white material shows long threads filled with coccoid bodies.

*Deep Agar.*—Anaërobic colonies grow slowly and in one to two weeks are the size of millet seeds. Yellowish-red and irregular, not homogeneous. Microscopically made up of threads 10 to 15 $\mu$ long irregularly stained and homogeneous short forms intensely stained. Ends of both rods and threads are swollen. Club-shaped forms are Gram-positive and may contain spores.

*Potato.*—No growth on the surface. In the glycerin water definite growth made up of long slender threads, swollen at one or both ends.

**Pathogenic Action.**—Very slight. Subcutaneous injection of guinea-pigs causes, occasionally, round hard tumor-like masses which gradually disappear.

Rodella: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1920, lxxxiv, 450.

### Actinomyces gypsoides Henrici and Gardner

Described originally by Henrici and Gardner from the sputum of a case of chronic bronchitis.

**Morphology and Staining Reactions.**—Acid-fast branching filaments in the sputum, the branches being lateral at irregular intervals. The mass of mycelium may show closely intertwined central filaments, the peripheral portions being arranged in a radial fashion somewhat resembling the sulphur granules of ordinary actinomyces. The filaments are usually uniform with occasional fusiform swellings. Definite clubbed extremities were never found. Many mycelial fragments also occurred of varying length, some showing a remarkable resemblance to the tubercle bacillus. The acid-fastness of the mycelium is not uniform, some strands taking the blue counterstain. The organisms are not so acid-fast as the tubercle bacillus, practically all the mycelium being decolorized by 5 per cent. hydrochloric acid-alcohol in a moment or two.

Colonies in glycerol agar appear in twenty-four hours as small white dots which under the microscope show the star-like structure described by Eppinger for *Actinomyces asteroides*. Subcultures from colonies grow readily, growth appearing over night.

**Cultural Characters.**—On agar the growth first appears as a thin grayish veil that soon becomes opaque, chalky-white in color, the surface being dry and wrinkled. The growth is firmly adherent to the medium and when touched with a platinum needle breaks off in large flakes. It is quite unlike the mealy material of *Actinomyces asteroides*. If the whitish bloom is scraped off the underlying mycelium is found to have a buff color, but is never orange or yellow like "asteroides."

Growth is more rapid and luxuriant on agar containing various carbohydrates such as dextrose, maltose, and glycerol but otherwise it is the same.

On potato growth is like that on agar.

Growth not so rapid on Dorset's egg medium or on Loeffler's serum. No liquefaction of these media.

Gelatin stabs show growth only on the surface. The medium is liquefied, digestion beginning in a few days and being complete in a week. The liquefaction is stratiform. In liquid media growth occurs only on the surface, appearing first as small floating white flakes. These soon coalesce and form a thick wrinkled snow-white pellicle turning up on the edges to extend a centimeter or more up the wall of the glass container. After about two weeks this pellicle breaks on jarring and falls to the bottom of the vessel after which no further growth occurs.

On litmus milk the pellicle is similar, with a somewhat yellowish tinge. The milk turns alkaline and is curdled in a few days. The litmus is reduced. The curdling is apparently due to a rennin-like ferment. The coagulated casein is slowly digested, peptonization being complete in about two weeks.

Carbohydrates not fermented. No diastatic action on starch plates.

No hemolysis on blood agar plates.

All meat extract media show a brownish discoloration after a time. This discoloration is more striking in Dunham's peptone solution. The colorless fluid just beneath the pellicle first assumes a straw color and then this color gradually diffuses through the medium and after some weeks becomes quite brown.*

The organism grows readily on such mediums as Czapek's agar containing only inorganic salts and saccharose, and agar made from an infusion of soil.

**Pathogenic Action.**—Intravenous injection of rabbits with broth cultures shaken till the pellicle is pretty thoroughly broken up produces death in forty-eight hours, with minute abscesses in various viscera but especially numerous in the kidney. This is studded with minute yellowish-white dots which bulge slightly above the cortex. On section these are also present in both cortex and medulla. Microscopically these abscesses are aggregations of polymorphonuclear leukocytes. Intraperitoneal inoculation of guinea-pigs produces death in five to six days with small tubercle-like nodules scattered over the peritoneal surfaces. On section these nodules prove to be small abscesses containing necrotic material and pus cells in the center and encapsulated by a zone of granulation containing mononuclear leukocytes and fibroblasts.

The virulence of the cultures remained undiminished for eight months.

An antigen prepared from the organism produced a severe constitutional reaction in the patient with a temperature of 102° F. but without any change at the point of inoculation. The complement-fixation test was strongly positive with the patient's serum, which also agglutinated the mycelial fragments in a dilution of 1 : 20 and partially at 1 : 100. Normal individuals gave no complement-fixation and no agglutination with the organism. Treatment of the patient with vaccines from the organism seemed to be beneficial.

Henrici and Gardner: J. Infect. Dis., 1921, xxviii, 232.

### Actinomyces Foersteri (Cohn)

Originally reported by Ferdinand Cohn as *Streptothrix Foersteri* in material from inflamed tear ducts given him by Foerster. Fine thin entangled threads surrounded by masses of micrococci. Some twisted and branching and thus differentiated from Leptothrix which is always rigid and unbranched. According to Silberschmidt two varieties of streptothrix (actinomyces) may be cultivated from the tear-duct, one a facultative anaërobe and one an obligate anaërobe.

Silberschmidt: Ann. de l'Inst. Pasteur, 1899, xiii, 841.

* This reaction has been explained by Beijerinck as being due to the formation of quinone and is associated with the fermentation of tyrosin.

### ACTINOMYCES AUREA (Saint-Sévérin)

Found by Du Bois Saint-Sévérin in conjunctivitis. Growth on all media. Liquefaction of gelatin, powdery spores. Gray and moist on serum, yellow wrinkled colonies on potato. Thickness of threads about 1 $\mu$. Aërobic. Non-pathogenic. Named *Streptothrix aurea* by Saint-Sévérin and regarded as possibly identical with Cohn's organism.

Saint Sévérin: Arch. de méd. nav., 1895. p. 252.

### ACTINOMYCES RUBRA (Kruse)

Isolated by Ruiz Casabo[1] from sputum.

Same as *Streptothrix rubra* of Kruse.[2]

Thick branching threads with spores. Rapid development, also under anaërobic conditions, with formation of brick-red colonies on the surface. Gelatin liquefied but without pigmentation. Not pathogenic.

1. Ruiz Casabo: Centralbl. f. Bakteriol. [etc.], 1894, xvii, 466.
2. Kruse: Die Mikroorganismen (Flügge). 3. Aufl. 1896, ii, 63.

### ACTINOMYCES METCHNIKOVI (Sauvageau and Radais)

Described as *Oöspora Metchnikovi* by Sauvageau and Radais from gelatin plates seeded with conduit water.

**Morphology.**—Branching threads about 0.3 $\mu$ in diameter retaining Gram's stain. Marked segmentation especially on organisms from the surface of cultures. No spores.

**Cultural Characters.**—*Gelatin Stab.*—Colonies in two to three days, small, globular and whitish along line of puncture with a grayish button on the surface which gradually becomes indurated and brown. Gelatin liquefied in fifteen to twenty days assuming a brown color.

*Potato.*—Growth slow. Grayish or brown irregular fissured colonies after several days at 35° C. About the colonies the agar gradually becomes brownish-black.

*Agar.*—Small isolated grayish colonies.

*Glycerin Agar* (4–8 per cent.).—Colonies rose-violet with a brownish pigmentation of the agar.

*Milk.*—Growth in the cream layer as a brown pigmentation gradually becoming rose-brown. Reaction acid at first, then alkaline. No coagulation.

Sauvageau and Radais: Ann. de l'Inst. Pasteur, 1892, vi, 242.

### ACTINOMYCES GUIGNARDI (Sauvageau and Radais)

Originally reported by Sauvageau and Radais as *Oöspora Guignardi.*

**Morphology.**—Long tangled branching filaments and spherical or ovoid spores easily stained by Gram's method and larger than the diameter of the filaments.

**Cultural Characters.**—*Agar Slant.*—Uniform expansion with transverse striations—vermiform. At the end of three days the growth is covered with a chalky powder (due to conidia).

*Glycerin Agar* (4–8 per cent.).—Growth more abundant than on ordinary agar.

*Broth.*—Flocculent growth.

*Milk.*—Yellowish growth in the cream layer with formation of spores (conidia). The milk is gradually digested and looks like broth. Reaction at first acid, then alkaline.

Sauvageau and Radais: Ann. de l'Inst. Pasteur, 1892, vi, 242.

### ACTINOMYCES INVULNERABILIS Kruse

Found by Acosta and Grande Rossi[1] in river-water and as a contamination on media. Named *Streptothrix invulnerabilis* by Kruse.[2] Resistant to high temperatures 100° to 120° C.

Branching mycelium with air hyphæ. Egg-yellow hard colonies covered with white down, which coalesce to wrinkled skins or membranes. Gelatin

liquefied at room temperature and also under anaërobic conditions. On potato a black pigmentation in the neighborhood of the growth. Growth in water in the form of cloudy turbidity. Not pathogenic.

1. Acosta and Grande Rossi: [Abstr.] Centralbl. f. Bakteriol. [etc.], 1893, xiv, 14.
2. Kruse: Die Mikroorganismen (Flügge). 3. Aufl. 1896, ii, 64.

### Actinomyces flava Sanfelici

Cultivated frequently by Sanfelici on agar plates exposed to the air. Deep colonies made up of fine anastomosing threads. Superficial colonies round, yellow, refractile. Agar slants show isolated colonies which fuse and form a folded layer intensely yellow in color. Potato growth as a thick folded membrane, slightly less yellow than the agar culture. In old potato cultures the color varies, being white, citron-yellow, flesh-colored or orange-red.

Partially acid-fast, that is, some segments remain stained by Ziehl-Gabbet's method.

Pathogenic to rabbits and guinea-pigs by intravenous inoculation.

Death apparently due to protein intoxication.

Sanfelici: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1904, xxxvi, 355.

### Actinomyces chalcea (Foulerton)

Described by Foulerton as *Streptothrix chalcea* and isolated from the air.

Growth occurred freely on all ordinary culture media, better at 37° C. than at 22° C. On nutrient agar growth copper-colored, becoming deep reddish-brown with age. Gelatin liquefied slowly. Growth on potato deep red in color, free and of crystalline appearance. Growth in broth produced typical round flattened colonies. Growth in milk with deposition of a very fine sediment and gradual clearing. Thermal death-point between 60° and 70° C. Diastatic action on starch. Pathogenic action not tested.

Foulerton: Lancet, 1905, i, 1200.

### Caminiti's actinomyces

Described by Caminiti as a streptothrix and isolated from air plates.

**Morphology.**—Masses of thin threads in young cultures arranged in tangled masses. The individual threads are straight or slightly bent, and show true branching. In old cultures chiefly rod-shaped forms and granules due to the fragmentation of the threads.

*Staining Reactions.*—Stains well with ordinary anilin dyes by Loeffler's method, by Ziehl's carbolfuchsin and by Gram. Stained also by Ziehl-Gabbet's method, but does not retain the stain completely, *i. e.*, partially acid-fast. The granules arising from the threads may be stained by Moeller's spore stain.

Cultivated aërobically without difficulty. Growth less abundant under anaërobic conditions.

**Cultural Characters.**—*Agar Plates.*—Small punctiform colonies appearing in forty-eight hours and reaching maximum development in three to four days. They are white and look as if they were covered with lime dust when on the surface and are whitish-yellow in the depths. They vary in size from the head of a needle to twice that and are firmly adherent to the agar.

*Agar Slant.*—Similar white colonies which increase in size having a diameter of 2 mm. in five to seven days. They are round, raised, and firmly adherent to the agar. As the colonies get older they become folded and wrinkled with raised centers. They are opaque, white and look as if covered with lime dust.

*Agar Stab.*—Compact expansion on the surface with a few small isolated spherical opaque white colonies in the upper part of the line of inoculation.

*Glycerin Agar.*—Rapid and abundant development. On 2 per cent. glycerin agar dry

gray-white colonies raised from the medium and firmly adherent to it. The centers are raised and look as if covered with lime dust. On 8 per cent. glycerin agar round raised adherent colonies with fringed edges, about the size of hemp seed. Occasionally isolated colonies are larger, rusty-brown in color, darker in the middle than in the periphery. The medium gradually turns light brown. On 20 per cent. glycerin agar the colonies have the same peculiarities but are opaque and black.

*Sugar Agar.*—Colonies about the same as on plain agar but development more rapid, usually small and white. On slant sugar agar the colonies are small, opaque, white, and adherent to the medium.

*Gelatin Plates.*—Small opaque white colonies, about the size of hemp seeds, round and finely mottled, centers thicker than peripheries. Gelatin liquefied.

*Gelatin Stab.*—Superficial growth and isolated colonies in upper part of line of puncture. The gelatin is gradually liquefied and the colonies coalesce to a thick membrane, whose edges are irregularly raised and whose centers are hollowed out. The colonies may be grayish-white in color. Liquefaction never complete.

*Broth.*—Punctiform white hemispherical colonies on the surface in three to four days. These coalesce, new colonies developing in the periphery. Gradually a tenacious firm membrane is formed, at first white in color then darker. In the depths the colonies develop as white delicate flocculi. No turbidity in the broth.

*Serum Broth.*—More abundant development, the colonies having a grayish-white color.

*Glycerin Broth* (5 per cent.).—Rapid development, the colonies having a light rust color. The surface colonies coalesce to form a tough irregular membrane which sinks on shaking the tube, a new membrane developing grayish-white in color. With more glycerin, 5 parts to 5 or 6 broth, the development is more abundant, the colonies turning absolutely black and the broth becoming blackish but clear.

*Blood-serum.*—Opaque white raised colonies coalescing to an adherent expansion.

*Glycerin Serum.*—Opaque gray-green colonies, the medium turning pale violet.

*Milk.*—Thick greenish opaque folded surface membrane. Occasionally dark green or pale white. Flocculent coagulum sinking to the bottom, the milk remaining clear and assuming a pale rose or violet color. Reaction amphoteric.

*Potato.*—Abundant development of small colonies which coalesce to form a membrane with irregular edges. Color, white yellowish or greenish, rarely rose or straw yellow.

Inverting or diastatic enzymes produced but no emulsifying or coagulating enzymes.

**Pathogenic Action.**—Virulent for guinea-pigs rabbits, and dogs. Subcutaneous injections produce swellings the size of a hazel-nut, consisting of a thick capsule with yellowish granular contents the consistency of dough. The animals lose in weight but do not die. Intraperitoneal injections are fatal, the animals dying after progressive emaciation. At autopsy the visceral and parietal peritoneum are covered with numerous disseminated punctiform nodules, grayish-white in color and the size of a needle head. Intravenous injections also fatal. At autopsy similar nodules appear in the various organs, on section showing long threads and isolated rods with chains of branching threads. Regarded by the author as a new species differentiated from *Actinomyces violacea* and *Actinomyces viridis* by its being only partially acid-fast, these two latter being completely acid-fast.

Caminiti: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1907, xliv, 193.

### ACTINOMYCES VIOLACEA (Rossi-Doria)

Isolated frequently from air and water by Rossi-Doria.[1] Description from Kruse.[2] Typical streptothrix with branching mycelium and sporulating aerial threads. Gram-positive.

Colonies violet, at first isolated, then combined in folded membranes. The medium also is colored red-violet.

*Gelatin* liquefied at ordinary temperatures.

*Bouillon* shows a very slow development of compact nodules at the bottom, isolated colonies on the surface. The fluid turns pale wine-red.

*Potato.*—Red-violet colonies, covered with whitish down and with a brown color in the vicinity.

*Milk.*—Violet nodules and very slow peptonization.

No aërobic growth.

Usually non-pathogenic. On one occasion intraperitoneal injection of 2 c.c. produced in twenty-two days a pseudotuberculosis of the mesenteric glands, spleen, liver, and lungs.

1. Rossi-Doria: Ann. d. Ist. d'ig. sper. d. Univ. di Roma, 1891, i, 399.
2. Kruse: Die Mikroorganismen (Flügge). 1896, ii, 62.

### ACTINOMYCES AURANTIACA (Rossi-Doria)

Originally isolated by Rossi-Doria from the air. Description from Kruse.

Branching threads with aërial mycelia and spores. Grows at ordinary temperature, not anaërobically.

Colonies in *gelatin* like drops of wax, pale yellow, then orange-colored, finally covered with white down.

No aërial mycelium on *agar*.

Colonies coalesce and form a warty membrane, not folded.

*Gelatin* not liquefied. No pigment on the surface.

*Potato* shows a thin pellicle gradually assuming an orange color.

Orange-colored flecks on *milk* which is otherwise not changed.

Non-pathogenic.

### ACTINOMYCES CITREA

Named but not described by Gasperini.

Gasperini: Centralbl. f. Bakteriol. [etc.], 1894, xv, 684.

### ACTINOMYCES CARNEA Rossi-Doria

Found commonly in the air by Rossi-Doria.
Description from Kruse.

Branching threads with aërial mycelium and spores. Growth at ordinary temperature. Colonies on all media small with a rosy color, the medium itself being colored red.

Membranes of growth may be warty but are never folded.

Gelatin not liquefied.

Non-pathogenic.

### ACTINOMYCES CHROMOGENES (Gasperini)

Same as *Streptothrix nigra* Rossi-Doria.
Description from Kruse.[1]

Frequently isolated from the air. Also found by Kruse and Pasquale[2] in the mesenteric glands of a patient dead of dysentery and also in a liver abscess. Branching threads with abundant aërial mycelium, which seems to fail only on glycerin agar.

Rapid development at ordinary temperature, only as an aërobe.

Colonies warty, frequently torn in the middle, dirty yellow, covered with white down and oil drops.

Media colored deep brown.

*Gelatin* liquefied.

*Milk* peptonized and covered with a membrane.

*Broth* covered with a membrane.

Grows very abundantly on vegetables, slowly in water.

Not pathogenic.

1. Kruse: Die Mikroorganismen (Flügge). 3. Aufl. 1896, ii, 63.
2. Kruse and Pasquale: Ztschr. f. Hyg., 1894, xvi, 1.

### ACTINOMYCES ALBIDO-FLAVA (Rossi-Doria)

Found frequently in the air by Rossi-Doria.
Description from Kruse.

Branching threads with sparse aërial mycelium. Growth at ordinary temperature, not as an anaërobe.

Colonies yellowish, media not colored. Growth superficial, furrowed, and ridged.

Slow liquefaction of gelatin.

Abundant growth in the form of swimming islets in milk which is peptonized.

Non-pathogenic.

### ACTINOMYCES ALBA (Rossi-Doria)

The most common actinomyces or streptothrix in air and water.

Branching threads with very abundant aërial mycelium which gives a whitish appearance to all cultures.

Growth aërobic. When gelatin colonies coalesce they form a large broadly-folded membrane with white down and oil droplets. Gelatin liquefied. Milk peptonized. Abundant growth on vegetables of all kinds.

Non-pathogenic.

### ACTINOMYCES THERMOPHILUS Gilbert

Originally obtained by Gilbert by inoculating potato with garden earth and incubating it at 55° C. In twenty-four hours a salmon-yellow or yellow color appears. In forty-eight hours white warty points show on this pale background and in a short time these fuse to a uniform white expansion of greater or less extent. The growth penetrates the potato to a depth of 2 mm. and rises above the surface to the same extent. The growth is folded and wrinkled. On the third day the white expansion appears flecked with particles of gray dust here and there. In four days the white mass has become pale gray. This expansion is surrounded by a pale yellow zone, 3 to 4 mm. wide. (Two of Gilbert's isolations turned coal-black later.)

**Morphology.**—The first yellow growth on potato consisted of a mycelium of long homogeneous rods showing true branching, 0.5 to 0.6 $\mu$ in thickness. The chalky growth contained originally shorter more refractile threads, made up of spores arranged in chains like chains of pearls. These spores have a diameter of 0.8 to 1 $\mu$. As the culture ages, changing from white to gray, more spores develop on the surface while the mycelium in the depths and in the pale yellow peripheral zone splits up into short bacillary and coccus-like elements. The morphology is best brought out by hardening the potato in alcohol, embedding in paraffin and staining with Loeffler's methylene-blue and Gram. The threads lying free retain Gram's stain better than those in the depths.

**Cultural Reactions.**—At temperatures above 55° C. development is slower. It ceases entirely above 60° C. Growth at lower temperatures, down to 45° C., but growth at 37° C. only with a few strains and then sparse.

No growth on potato under 35° C.

Spores formed abundantly on slightly alkaline fruit juices.

*Plain or Glycerin Agar Plates.*—Within twenty-four to forty-eight hours round, feathery colonies appear, salmon-yellow in color and consisting of dark centers with a light zone of threads extending in all directions. After two to three days the center of the colony is covered with a moist glistening drop which appears silver-gray under the microscope. Colonies about 3 mm. in size. They coalesce when thickly seeded.

*Plain or Glycerin Agar Slants.*—At 55° to 57° C. in twenty-four to forty-eight hours pale yellow colonies 3 to 4 mm. in size, centers somewhat down-like. In a few days these colonies become 1 cm. in diameter and show radially arranged folds. When the seeding is heavy the colonies coalesce to a coherent even tough membrane which later shows many folds.

Spores rarely formed on agar.

*Glucose or Lactose Agar.*—Abundant development of colonies coalescing to a tough membrane. On 2 per cent. glucose agar spores formed abundantly after two and one-half months. The membrane of growth is covered here and there with whitish-gray powder. The same appearance is seen on 5 per cent. glucose agar in three to four weeks. No fermentation of sugar.

*Gelatin Stab.*—After three days pale yellow small round colonies, more abundant near the surface and gradually forming a trough-like excavation. Development sparser near the bottom. The growth suggests an inverted fir-tree. Liquefaction begins in about six days but does not reach a high point.

*Blood-serum.*—Folded membrane about the color of the medium, sinking into the liquefied serum in about eight to fourteen days.

*Broth.*—In twenty-four hours at 55° C. pale yellow delicate round floccules of clearly radiate structure, about 2 mm. in diameter. When only a few colonies develop, they are two to three times this size and show their strands clearly to the naked eye. No turbidity. Somewhat less development at 37° C.

*Milk.*—No change at first at 37° C. After forty-eight hours a tough orange-yellow crust

on the surface consisting of long threads. After four to five days coagulation begins. Reaction becomes acid in eight days, 1 c.c. filtered milk neutralizing 0.25 to 0.6 c.c. of 1 per cent. normal sodium hydrate.

Cultures give off a fruit-like odor, which becomes mold-like when the spores develop. Non-pathogenic to rabbits and guinea-pigs.

Gilbert: Ztschr. f. Hyg., 1904, xlvii, 383.

## GENUS 4. ERYSIPELOTHRIX Rosenbach

Rod-shaped organisms with a tendency to the formation of long filaments which may show branching. The filaments may also thicken and show characteristic granules. No spores. Non-motile. Gram-positive. Do not produce acid. (Microaërophilic.) Usually parasitic.

Type species *Erysipelothrix rhusiopathiæ* (Loeffler).
*Bacillus murisepticus* (Loeffler).

### ERYSIPELOTHRIX RHUSIOPATHIÆ (Loeffler)

The etiological agent of swine-erysipelas (Rotlauf), usually known as *Bacillus erysipelatos-suis* or *Bacillus rhusiopathiæ-suis*, was first observed and cultivated by Loeffler[1] whose work was confirmed and extended by Schütz[2] and by Lydtin and Schottelius.[3] (Description from Preisz.[4])

**Morphology.**—In the tissues the organisms are small, vary in size and are three to four times as long as broad or even ten times. They are often in pairs end-to-end and are straight or slightly curved. They are frequently found in the leukocytes where they may be demonstrated by picrocarmin followed by Gram's stain. In cultures the organisms also vary in size and are much longer and thicker both on solid and in fluid media. Long threads frequently develop which show true branching. Involution or teratological forms, large and swollen, on media containing saponin (Sabella[5]).

No spores. Bright areas frequently are visible in the stained organisms but they are not spores. Non-motile.

**Staining Reactions.**—Stains best with aqueous anilin dyes, fuchsin, and gentian-violet. Gram-positive.

**Cultivated** with a certain amount of difficulty, the best growth being on gelatin and under anaërobic conditions.

*Plain Agar Plates.*—Colonies appear in twenty-four hours at 37° C. as fine bluish transparent areas like drops of dew, barely visible to the naked eye. On crowded plates they do not increase in size. When sparsely seeded they grow larger but never more than ½ mm. in diameter. They are thin with smooth edges and show structureless centers and conical peripheries.

*Gelatin Plates.*—At room temperature characteristic colonies appear in a few days, small delicate floccules with white centers and without sharply marked contours. On fairly thickly seeded plates they are not over 1 mm. in size. Under low power, 30 to 50 diameters, the center of the colony appears sparsely and irregularly granular, the periphery being made up of a tangle of irregularly curved threads of different thicknesses which penetrate the media. The threads often show granular or knob-like thickenings. With plates less thickly seeded colonies after a few weeks are 2 to 3 mm. in size, delicate bluish transparent clouds with indented edges somewhat resembling cotton wool. The colonies often appear sunken.

*Gelatin Stab.*—Growth varies from small white points along the line of inoculation to well-developed bluish almost structureless clouds. Often small whitish points develop along the stab from which at various places cloudy peripheral processes penetrate the gelatin. Generally the growth appears as small fine floccules like snowflakes along the stab, with lateral clouds passing into the gelatin in various directions. No liquefaction but occasionally some softening. Growth takes place both at the top and the bottom of the stab and a small air bubble may appear at the beginning. On the surface there is a barely visible delicate expansion.

*Gelatin Slant.*—Bluish-white scale-like transparent colonies, centers raised and uneven, edges thin and jagged. Diameter seldom more than 2 mm.

*Bouillon.*—Moderate turbidity reaching its maximum in one to two days at 37° C. No superficial film or flocculi. The fluid clears, the organisms sinking to the bottom where they form a coherent viscid mass which can be dislodged only by powerful shaking. Flask cultures develop an intense fetid odor in the first few days. More abundant growth in 0.1 per cent. sheep blood bouillon.

*Dextrose Bouillon.*—Growth more abundant than in plain bouillon. The culture develops acid and according to Gordon becomes avirulent for mice.

*Potato.*—No growth.

**Resistance** fairly high. Dried on various objects and kept in the thermostat at 37° C., the organisms are viable after one month, in direct sunlight viable after twelve days, in 20 per cent. Pearson's kreolin after twenty-four hours (Sirena and Alassi). Destroyed in fifteen minutes at 70° C., sometimes at 52° C. When present in meat of infected swine they are not destroyed by two and one-half hours roasting or stewing, but are killed by boiling the meat. The organisms die out slowly in salted meat but may be present after a month. In smoked hams virulent organisms may be found after three months but not after six months. In buried carcasses of animals dead of swine-erysipelas the bacteria may live two hundred and eighty days (Lösener[6]).

**Pathogenic Action.**—Freshly isolated cultures are virulent to white and gray mice, wood mice, pigeons, rabbits, and swine. Mice develop a conjunctivitis in which the eyelids are fastened together with the secretion. They die in three to four days, rarely in one to two days. Pigeons die in three to six days. In rabbits direct injection into the ear produces an erysipelas-like inflammation which slowly heals. Intravenous injection kills them in three to six days. Swine may be infected by subcutaneous inoculation. The organisms have no effect on field-mice, guinea-pigs, dogs, cats, hens, geese, ducks, horses, asses, mules, cattle, and sheep. They have no effect on man when present in the alimentary canal, but may produce lesions on the hands in butchers and veterinarians. These rarely last some weeks and are accompanied by red patches, painful and tender, and swelling of the adjacent glands. Rosenbach has reported 8 cases of human infection.

Infection also produced by feeding swine infected organs (Pasteur, Lydtin, and Schottelius), but not by feeding cultures. Preisz infected five to six weeks old pigs by scarifying the skin and rubbing in broth cultures recently obtained from infected cadavers.

**Virulence.**—Cultures vary greatly in virulence and lose it rapidly. Thus broth cultures of which small quantities, 0.001 to 0.002 c.c., kill pigeons in three days at one time, after a few weeks either fail to kill in large doses or kill only after six to eight days. Virulence is lost rapidly in artificial cultures. It may be restored by passing through pigeons. By passing the organism repeatedly through rabbits, Pasteur found that the virus became stronger for rabbits and weaker for swine, by passing it through pigeons it became stronger for swine. Passage through swine increases its virulence for swine so that it kills swine in forty-eight hours.

**Distribution.**—The organisms of swine-erysipelas may be found in large numbers in the dejecta of swine (Cornevin and Kitt), in the colon and cecum (Olt), in the tonsils (Bauermeister). Pitt found them in the intestine twenty-six times and in the tonsils twenty-eight times in 66 examinations. Virulent organisms were also found by Pitt in the gall-bladder of recovered swine.

**Vaccines** were first prepared for protective inoculation against swine-erysipelas by Pasteur. Prophylactic inoculation against the disease is practiced in France, Russia, and Germany with favorable results. Animals can be actively immunized against the disease, and their serum has been used on a large scale to produce passive immunity. A combination of vaccination and serum treatment is also largely employed.

1. Loeffler: Arb. a. d. K. Gsndhtsamte, 1886, i, 46; 141.
2. Schütz: Arb. a. d. K. Gsndhtsamte, 1886, i, 57.
3. Lydtin and Schottelius: Rotlauf der Schweine. Wiesbaden, 1885.
4. Preisz: Rotlauf der Schweine. *In:* Handb. d. pathog. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, vi, 1.
5. Sabella: Centralbl. f. Bakteriol. [etc.], 1925, xciv, 411.
6. Lösener: Arb. a. d. K. Gsndhtsamte, 1896, xii, 448.

### Bacillus murisepticus Loeffler

Described originally by Koch[1] as the "Bacillus of mouse septicemia" and obtained by him by injecting mice with putrefying infusions. An epidemic due to this bacillus was subsequently observed by Loeffler.[2] Cultivated by Johne from decomposed meat, and by Preisz from foul beef blood.

The organism closely resembles the bacteria of swine-erysipelas. Preisz[3] states that it is smaller in the blood of infected animals, while the latter organisms are thinner in cultures. On gelatin plates *Bacillus murisepticus* produces colonies showing fine branches and threads radially arranged, the outermost threads terminating in delicate spirals. These differ from the colonies of *Bacillus rhusiopathiæ suis*. In gelatin stabs growth is more rapid and occasionally concentric zones appear. This growth also differs from the gelatin growth of swine-erysipelas.

**Pathogenic Action.**—*Bacillus murisepticus* is almost without virulence for swine. If cultures be rubbed on the skin of shoats an erythema results but no infection. Small animals are sensitive to the organism just as they are to swine-erysipelas. White and gray mice and pigeons are easily infected. Rabbits are somewhat sensitive. Large animals are resistant.

**Distribution.**—This organism is apparently widely distributed in nature, especially in putrefying materials.

**Remarks.**—The exact relationship of *Bacillus murisepticus* to *Bacillus rhusiopathiæ suis* is not clear Lorenz has immunized rabbits and swine against the virus of swine-erysipelas by *Bacillus murisepticus*. Preisz, Jensen,[4] and Prettner[5] regard the organisms as the same.

1. Koch: Wund Untersuchungen ueber de Aetiologie der infektionskrankheiten. Leipzig, 1878.
2. Loeffler: Mitt. a. d. K. Gsndhtsamte, 1881, i, 134.
3. Preisz: Rotlauf der Schweine. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2 Aufl., 1913, vi, 1.
4. Jensen: Deutsche Ztschr. f. Tiermed., 1890–91, xvii, 295.
5. Prettner: Berl. tierärztl. Wchnschr., 1901.

## FAMILY II. MYCOBACTERIACEÆ Chester

Usually parasitic. Rod-shaped, frequently irregular in form, but rarely filamentous and with only slight and occasional branching. Often stain unevenly (showing variations in staining reaction within the cell). No conidia.

### GENUS I. MYCOBACTERIUM Lehmann and Neumann

Slender rods which are stained with difficulty, but when once stained are *acid-fast*. Cells sometimes show swollen, clavate or cuneate forms, and occasionally even branched cells. Non-motile, *Gram-positive*. No endospores. Growth on media slow. Aërobic. Several species pathogenic to animals.

Type species *Mycobacterium tuberculosis* (Koch) Lehmann and Neumann.

" " human type (Koch).
" " bovine type (Smith).
" *avium* (Straus and Gamaléia).
" *tropidonatum* (Sibley).
" *piscium* (Dubard and Terre).
" *chelonei* (Friedman).
" *ranæ* (Rupprecht and Küster).
" *lepræ* (Hansen).
" of rat leprosy (Stefansky).
" *paratuberculosis* (Jones and Frothingham).
" of Olschanetsky.
" *smegmatis* (Alvarez and Tavel).
" *berolinense* (Petri-Rabinovitsch).
" *phlei* (Moeller).
" *stercussis* (Moeller).
" *graminis* (Moeller).
" *rubropertinctum* (Grassberger) Hefferan.
" *friburgense* (Korn).
" *lacticola and friburgense* (Korn).
" *pseudoperlsucht* of Moeller.
" *alluvialum* (Kersten).

#### MYCOBACTERIUM TUBERCULOSIS (Koch) Lehmann and Neuman

Discovered by Koch[1] in spontaneous tuberculosis processes in man, cattle, horses, swine, goats, sheep, chickens, and apes, and in rabbits and

guinea-pigs artificially infected with tuberculous material. Named by Koch *Bacillus tuberculosis*. The organisms were originally demonstrated by special staining methods and subsequently cultivated on coagulated beef serum. Within a few days after Koch's publication, Baumgarten[2] reported that he had demonstrated rod-shaped bacteria in sections of tubercles of rabbits inoculated with cattle tuberculosis (Perlsucht) by treating the sections with potassium hydrate,, but was unable to stain them. He regarded them as the etiological agents in tuberculosis, however.

*Types of Tubercle Bacilli.*—Following the work of Koch it was generally believed that the organisms producing tuberculosis in man and in animals, especially cattle, were the same. It was pointed out by Theobald Smith in 1895 and 1896 that the tubercle bacilli from cattle differed markedly from those from other sources in pathogenic action, being much more virulent for nearly all animals, particularly for cattle. Later he pointed out certain distinguishing features in morphology and in cultural reactions chiefly as regards their growth in broth. Soon after Adami called attention to the constant differences in pathogenicity between the tubercle bacilli of human and bovine sources. Finally Koch announced at the International Congress on Tuberculosis in London that the human and bovine tubercle bacilli could be regarded as essentially different organisms. This differentiation between the tubercle bacilli of human and bovine sources has now been abundantly confirmed and painstaking investigations have been made in regard to the two types.

At present, therefore, we differentiate two types of mammalian tubercle bacilli, the human type and the bovine type, and the avian type in birds. In addition we have the acid-fast organisms found in cold-blooded animals related to but distinct from the preceding.

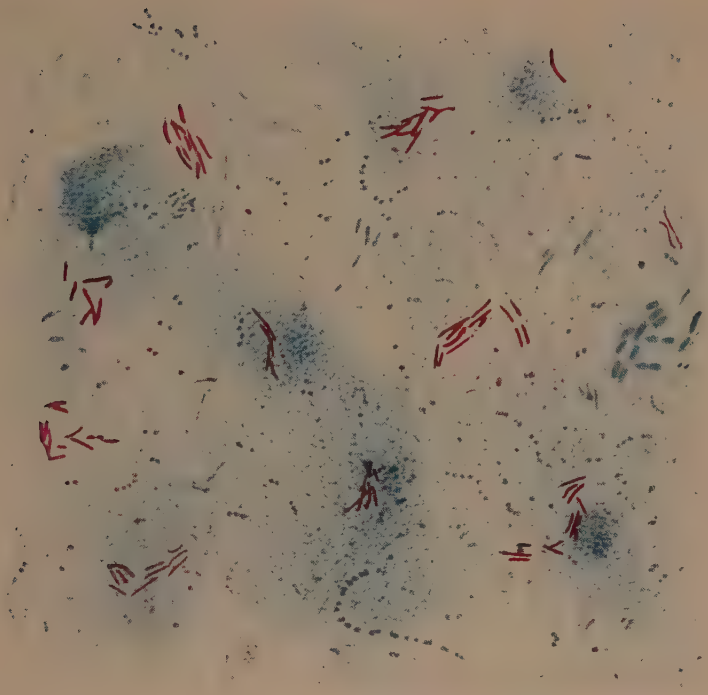
We may first consider the reactions which the human and bovine types of tubercle bacilli possess in common and then discuss their distinguishing features.

**Morphology.**—The unstained organisms were originally described by Koch as fine short non-motile rods with fine granules present in many individuals. The stained organisms were rods of about one-quarter to one-half the diameter of a red blood-corpuscle in length, of fairly constant diameter. They were usually slightly bent or curved, the long forms being almost spirals. They lay either within or without the cells in smaller or larger clumps. Eastwood[3] finds that the average length of the organism in tissues varies from 1.23 to 4.12 $\mu$, the shortest being 0.5 $\mu$, the longest 8 $\mu$. In cultures on horse serum the average length varies from 0.63 to 1.3 $\mu$, the shortest being 0.5 $\mu$, the longest 4 $\mu$.

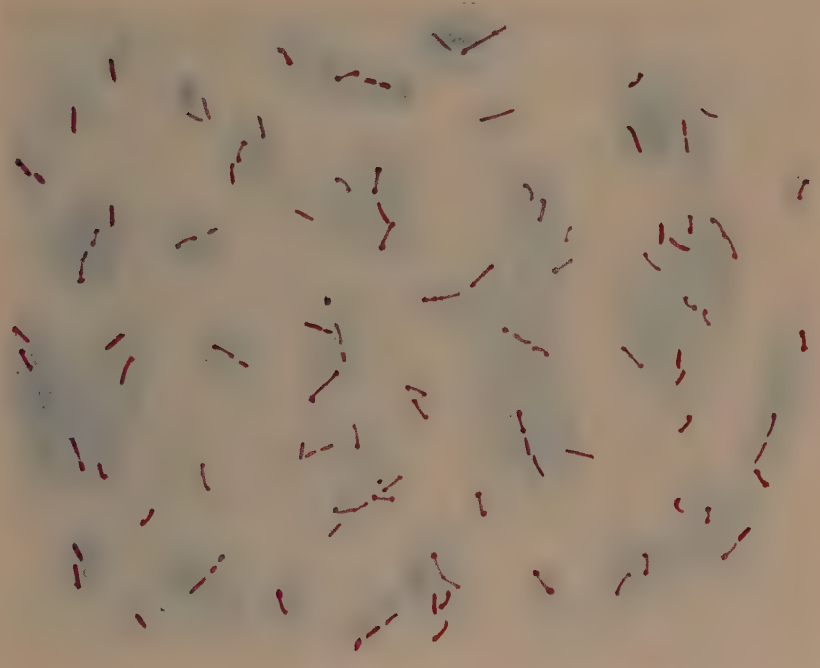
**Staining Reactions.**—The tubercle bacillus cannot be stained by ordinary methods. It was originally demonstrated by Koch by aqueous alkaline methylene-blue (1 c.c. concentrated methylene-blue solution in 200 c.c. distilled water with 2 c.c. 10 per cent. potassium hydrate added), counterstained with aqueous vesuvin. Subsequently a great many different stains for the organism have been devised among which the most important are those of Ehrlich, Ziehl-Neelsen, Gabbet, Gasis, Yamonoto, Martin, etc. These stains rest on the fact that the tubercle bacillus takes up stains with difficulty but when once stained retains the dye when exposed to alcohol and weak acid solutions. In virtue of this property they are called *acid-fast*, the acid-fastness resulting from the presence of certain chemical substances in the organism. Many of the organisms in the tissues and cultures are not acid-fast and lose the stain by these methods. The tubercle bacillus retains Gram's stain which brings out different morphological features. The organisms appear as chains of granules or small spherical elements, instead of as fairly regular and homogeneous rods. The original Gram stain has been modified for the tubercle bacillus by Much, Wehrli, Knoll, Weiss, and others.

*Granules.*—These were first observed by Koch and may be found in some of the organisms in almost any preparation. They were first regarded as spores and are thought to be spores or spore material by a number of authors, von Betegh[4], Knoll[5], and Gasis[6] among others. They have been especially studied by Ehrlich[7], Nocard and Roux[8], and by Metchnikoff.[9] It has been shown by Babes and Cornil[10] that they behave differently toward certain dyes than the rest of the cell protoplasm, appearing red in a blue background when the preparation

PLATE I



Tubercle bacillus from sputum, stained by carbolfuchsin and Gabbet's methylene-blue.



Tubercle bacillus, Koch I, from culture, stained with carbolfuchsin and Gabbet's methylene-blue.

is treated with anilin water fuchsin followed by intense staining with methylene-blue, and by Czaplewski[11] who stained them red by hot carbolfuchsin, decolorizing with sodium bisulphite and counterstaining with carbol methylene-blue. Granules are also brought out well by Much's modification of the Gram stain.

It is now usually believed that these granules represent reserve material of special chemical composition like the granules in other bacteria. There is no evidence that they possess any special resistance to heat or disinfectants. They may belong to the fat-like or lipoid constituents of the bacterial protoplasm. The tubercle bacillus does not produce true spores.

**Aberrant Forms.**—In addition to the usual rods found in tuberculous material and in cultures many observers have called attention to long threads and true branching. Thus Nocard and Roux observed lateral branching and a terminal swelling of the rods, while Metchnikoff reported branched threads with club-shaped swollen ends in cultures on glycerin agar. They have been especially investigated by Babes, Bruns[12] and Coppen-Jones[13] and by Craig,[14] Dorset,[15] and Wolbach and Ernst[16] in this country. Branching forms are common in the avian tubercle bacillus (Mafucci[17]) and in tubercle bacilli (Fischel[18]) from cold-blooded animals; it has been suggested that the cultures with branching are not human or bovine strains. Many of these observations have been made, however, on definite human tubercle bacilli (Craig) and on bovine bacilli (Dorset). Branching forms are common in old cultures but Wolbach and Ernst report them in comparatively young growths. The tubercle bacillus sometimes exhibits peculiar club-shaped terminal bodies which are strongly acid-fast. They have been observed especially by Coppen-Jones who places the tubercle bacillus among the actinomyces. They have been produced experimentally by Babes and Levaditi[19] by the injection of human tubercle bacilli under the dura of rabbits and by Friedrich[20] by injection into the left ventricle. They consist of rosettes of clubs like those seen in actinomyces and may be stained by eosin. Similar appearances have been noted by Abbot and Gildersleeve[21] in rabbits injected intravenously with other acid-fast bacteria.

Park and Williams[22] believe that the tubercle bacillus has a thin capsule, appearing thicker when stained with fuchsin than with methylene-blue.

**Methods of Isolation.**—The tubercle bacillus may be cultivated from tuberculous tissues in which it is the only organism present on a variety of media such as Koch's blood-serum, Loeffler's serum, glycerin agar, Dorset's egg medium, Petroff's gentian-violet medium, etc. It grows slowly, usually only at 37° C., colonies appearing only after about eight days. After artificial cultures have been established the organisms may be cultivated without difficulty on a variety of special media. When mixed with other bacteria, as in sputum, they can be isolated by the method of Kitasato,[23] by digesting the sputum in sodium hydrate (Mühlhäuser[24]), by Petroff's method[25] or by Uhlenhuth's antiformin method.[26]

**Cultural Characters.**—*General.—Coagulated Beef Serum with or without Glycerin.*—Isolated colonies or a coherent growth may appear in six to eight days as a fine dry expansion which gradually develops into a membranous layer. In two to three weeks the growth spreads over the surface, develops folds, and often climbs up on the wall of the container, or forms a membrane on the water of condensation. The water of condensation remains clear except for powdery or crumbling masses in the depths. The serum is not liquefied. The growth has a peculiar stiff crumbly consistence and is easily lifted by a platinum needle in delicate scales. The edge of the growth shows wave-like indentations caused by the disposition of the organisms in S-shaped rows.

The addition of glycerin to the serum as first recommended by Nocard and Roux makes the serum more favorable for the growth of the organism. Many strains produce a yellow pigment in old cultures.

*Plain agar* is not ordinarily a good medium for the tubercle bacillus although growth does occur, as Koch has pointed out. The addition of glycerin as recommended by Nocard gives a valuable medium for the cultivation of the organism after it is isolated. For isolation it is not as good as serum media. The glycerin agar growth is dry and deeply folded, with flakes, thick irregular granules and warty protuberances. A good deal of color appears in the cultures which become yellowish and occasionally reddish. The best results are gotten with 2 to 3 per cent. glycerin (Vagedos), although Nocard and Roux recommended 6 to 8 per cent. The cultures when well grown give off a fruit-like aromatic odor.

*Dorset's Egg Medium.*—This is a very valuable medium for the tubercle bacillus and has been extensively employed in America and in England. Growth is like that on serum but somewhat more moist.

*Capaldi's medium,* agar containing yolk of egg, gives favorable results. According to Kossel it is the lecithin which is the essential constituent of the egg-yolk since lecithin added to agar makes a good medium.

*Glycerin Broth.*—This was originally recommended by Koch.[27] In a few days after inoculation when kept at 37° C. the particles of material increase in size and in their neighborhood a delicate veil-like or net-like expansion occurs. This consists of bacilli, only some of which are acid-fast. The mass of growth increases in breadth and thickness and often in two weeks the entire surface of the broth is covered with membrane. This gradually

wrinkles and folds and crawls up the sides of the glass container. In consistency it is stiff and crumbly. The rapidity of growth depends upon the particular composition of the broth employed and upon the type of tubercle bacillus under observation. The membrane is at first gray and transparent but as it increases in thickness it looks like opalescent glass and turns first white and then yellow. The cultures have a musty fruit-like odor. After about six weeks at 37° C. growth ceases and the superficial membrane sinks to the bottom. The broth itself remains fairly clear. The reaction of the broth is a matter of importance, better results being obtained when the original acidity of the meat infusion is not neutralized.

Dorset[28] recommends the addition of acid potassium phosphate to the broth instead of sodium chlorid.

*Milk* freed from cream and containing 2 per cent. glycerin is often a favorable medium for the tubercle bacillus. Acid is produced and the milk coagulated (Griffith).

Other media for the cultivation of the tubercle bacillus are Petroff's gentian-violet agar, Pawlowski's glycerin potato, Kühne's protein-free medium, de Schweinitz's synthetic medium, etc.

**Resistance.**—The tubercle bacillus has a greater resistance to deleterious agencies than other bacteria, owing possibly to its fatty envelop. The majority of bacilli in a preparation die in a few hours on desiccation, a few resisting however for months. It remains viable in water and sewage for weeks (Park and Williams). In sputum it may retain its viability for months. In the cadaver of tuberculous individuals it retains its morphology, staining reactions and vitality for long periods (fifteen months). It remains alive three months in favorable cultures. It resists dry heat at 100° C. for twenty minutes but is destroyed in forty-five minutes. In milk it is destroyed at 60° C. in twenty minutes, at 65° C. in fifteen minutes, at 70° C. in about five minutes, at 80° C. in one minute and at 95° C. in one-half minute (Park and Williams). Theobald Smith finds that it is destroyed in milk at about the same temperature as ordinary vegetative bacteria, at 63° to 65° C. in twenty to thirty minutes.

The resistance of the tubercle bacillus to disinfectants is marked. It is destroyed in sputum in six hours by 5 per cent. carbolic acid. In cultures 5 per cent. iodoform is required to kill the organisms. It is killed slowly by fumes of sulphur or formaldehyd, requiring the fumes of 4 pounds of burning sulphur to 1000 feet of air space or 10 ounces of formaldehyd for 1000 feet of air space.

In sputum it is destroyed by drying in a few minutes to several hours depending upon the thickness of the material. In fine powder it is destroyed in five to ten days by diffuse daylight. In expectorated sputum the organisms die rapidly when exposed to direct sunlight but remain alive for weeks when kept in the shade.

**Pathogenic Action.**—After the tubercle bacillus gains entrance to the body, chiefly by way of the respiratory and intestinal tract, its characteristic effect is the production of a tubercle. This is primarily a hard gray translucent mass of tissue, somewhat smaller than a millet seed. Microscopically a tubercle consists of a mass of concentrically arranged cells with "elongated vesicular nuclei, little chromatin and faintly outlined cell body" (MacCallum). These are the so-called epithelioid cells. The central area of the mass is usually but not always occupied by a giant-cell, a large cell containing a great number of nuclei. In the outer portions of the tubercle a few mononuclear lymphoid cells may be found and the entire tubercle is supported by a framework of reticulum with a certain amount of fibrin. The tubercle itself is microscopic in size and the structure called a miliary tubercle is usually made up of a number of true tubercles. The tubercle bacilli lie inside the giant-cell and the spaces between the epithelioid cells. The central portion of the tubercles soon degenerates and disintegrates with the formation of a mass of necrotic cheesy material. In addition to the tubercle, the growth of the organism leads also to the formation of diffuse granulation tissue consisting of scattered epithelioid cells, giant-cells, and true tubercles and this tissue also rapidly undergoes degeneration.

Various tubercles may coalesce forming large masses of tuberculous tissue. When these break down the surrounding tissues also become necrotic and eventually smaller or larger cavities are formed, especially in structures like the lungs. The necrotic tuberculous tissue is frequently invaded by other organisms, particularly the pyogenic cocci and masses of pus are formed. Apparently also true pus may arise from the growth of the tubercle bacillus alone.

To determine the presence of tubercle bacilli in suspected materials susceptible animals like rabbits or guinea-pigs are inoculated, usually beneath the skin. If the organisms are present, the adjacent lymphatic glands become enlarged, the animals lose weight and die in the course of a few weeks with tubercles scattered through various parts of the body. After intraperitoneal inoculation an extensive exudate develops between the layers of the peritoneum, the abdominal lymphatic glands are enlarged and contain characteristic tubercles. Direct injection into the pleura leads to an exudate with the formation of tubercles in the pleura and the introduction of the organisms into the bronchi by artificial insufflation is followed by enlargement of the bronchial lymphatic glands and the formation of tubercles in the tissue of the lungs.

Following the introduction of the tubercle bacilli into the body the organisms may be

carried to nearly any part of the body, especially by way of the blood and lymph channels and in consequence tubercles may develop in practically any organ or tissue.

**Distribution.**—The tubercle bacillus is probably the most widely distributed pathogenic organism known since the disease processes which it causes are of the greatest diversity of character and may attack nearly every organ of the body. Tuberculosis is wide-spread in temperate climates and is especially abundant in tropical countries. The disease affects especially the respiratory tract and infections of the lungs are usually due to the tubercle bacillus, less frequently infections of the pleura. The organism is the frequent cause of disease of the genito-urinary tract, both kidney and bladder being involved in the infection. Disease of both the male and female genital organs is commonly due to this organism, which may infect the ovaries and the Fallopian tubes in the female and the epididymis of the male. Glandular tuberculosis and bone and joint tuberculosis are also frequent, especially in children, as well as tuberculosis infection of the meninges of the brain and cord, and of the peritoneum. Lupus is now recognized to be a true tuberculosis of the skin.

Fig. 4.—Tubercle bacilli from culture R1, glycerin agar two months, stained with carbol-fuchsin and methylene-blue.

Nearly every species of animal is subject to tuberculosis, cattle and swine being especially sensitive. There are great differences in the susceptibility of the different species to the different types of organisms however. Thus man is sensitive to both the human and bovine type and certain types of disease in children like abdominal tuberculosis and cervical adenitis are usually due to the bovine bacillus. Joint and bone tuberculosis are generally produced by the human type, as well as tuberculosis of the meninges. Occasionally generalized infections in man are to be traced to infection by the bovine bacillus as in the so-called butcher's tuberculosis (Ravanel). Pulmonary tuberculosis in man is usually due to the human tubercle bacillus and not to the bovine. Cattle are sensitive to the bovine type of the organism and refractory to the human type. Swine are highly susceptible to the bovine bacillus. Among small animals both rabbits and guinea-pigs are very sensitive to the bovine organism and less so to the human. Wild animals kept in captivity are especially prone to develop tuberculosis.

In tuberculosis of the lungs in both man and animals the organisms are present in the sputum, sometimes in enormous quantities. They may be present in the dejecta in both tuberculosis of the intestine and in lung tuberculosis from swallowing. They are rarely present in the urine in disease of the kidney and bladder. When present in small numbers

they are best demonstrated by the inoculation of susceptible animals like guinea-pigs. The tubercle bacillus apparently does not survive for long periods in the environment. It may be recovered from the dust of rooms in which phthisical patients with open tuberculosis have been living and according to Brown, Petroff, and Heise is rarely present in water contaminated by the discharges of tuberculous patients. The bovine bacillus is widely distributed in the milk of animals with outspoken disease of the udder and is occasionally present in the milk of animals with disease in other parts of the body. Occasionally milk is contaminated mechanically by individuals with open tuberculosis and in such cases it may contain the human tubercle bacillus. The tubercle bacillus may also be present in market meat from animals with tuberculosis of the organs. The muscles of animals are seldom invaded by the organism and the meat is generally contaminated mechanically.

**Special Characteristics of the Human Type.**—On serum with or without glycerin the human type forms longer and more slender organisms than the bovine. According to Wolbach and Ernst the length is 2 to 3 $\mu$ compared with 1 to 2 $\mu$ for the bovine. In glycerin meat-juice broth they are uniform in length and frequently curved. They stain well in cold carbolfuchsin and usually uniformly.

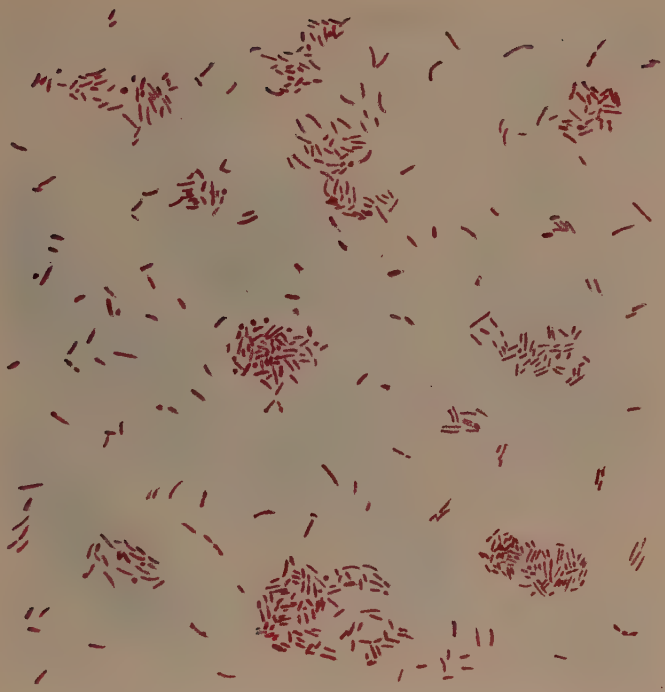
Cultivation not usually difficult. The organism grows well on the media already mentioned. Called "eugonic" by the English Commission. Bovine type called "dysgonic." According to Dorset round raised colonies are formed on egg media, or a dry expansion which clings to the surface. According to Park and Krumwiede the addition of glycerin favors the growth. On glycerin broth the organisms multiply in the first few days and within three weeks form a superficial membrane increasing in thickness, of brittle consistency which covers the whole surface and climbs up the side of the glass. Later this is folded and wrinkled. On potato the human type develops a yellow-red or orange-red pigment, not produced by the bovine type.

In Theobald Smith's broth the human type lessens the acidity in the first two weeks only. After this the acidity increases and in four weeks the original acidity is restored or passed (Smith[29]).

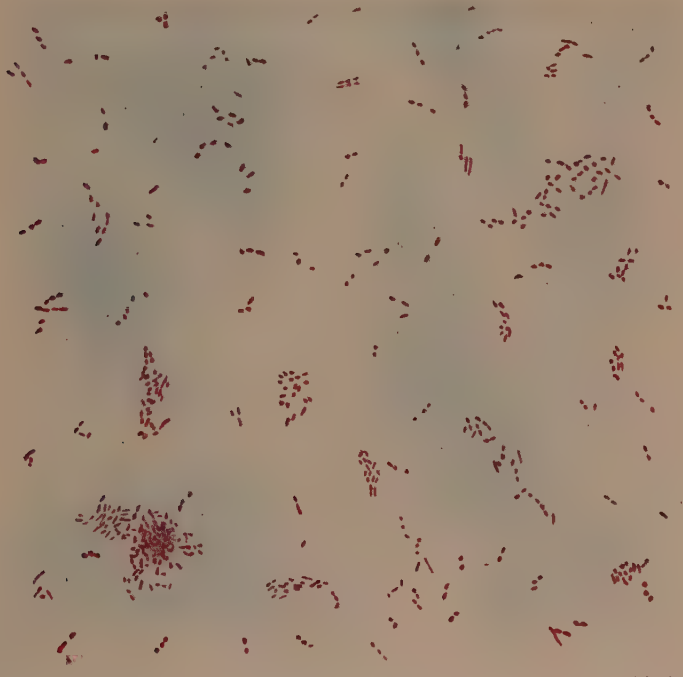
Pathogenic usually to guinea-pigs but only irregularly virulent for rabbits by subcutaneous or intravenous injection. Rabbits are more sensitive to intraperitoneal injection. Cattle are very resistant to intravenous and intraperitoneal inoculation and succumb only to large doses. Subcutaneous injection produces only localized abscesses. Calves fail to develop tuberculosis from feeding the human type. Inhalation infection also fails in cattle with the human type. Sheep and swine are not sensitive to subcutaneous injection. Young pigs develop tubercles in glands from feeding over a long time (de Schweinitz, Dorset and Schroeder, English Commission). Goats are slightly sensitive, cats not sensitive. Apes are very sensitive. Gibbons sensitive to both human and bovine types. (V. Dungern.) Canaries and parrots are both sensitive to human type.

**Special Characteristics of the Bovine Type.**—Organisms of the bovine type on beef serum are shorter, plumper, often oval cocci about 1 $\mu$ in length. They take the stain by Ziehl-Neelsen's method and can also be stained easily by cold carbolfuchsin (Smith, Spengler). In glycerin broth they vary in length from short coccus-like forms to medium and long bacilli. In the long organisms are deeply stained granules of varying size, round or oval, either in the middle or at the ends. Growth on serum slow. Called "dysgonic" by the English Commission. On Dorset's egg medium growth in small flat moist glistening colonies which tend to coalesce. These colonies are easily broken up in broth. The addition of glycerin to the medium hinders rather than favors the development. In glycerin broth the growth is a delicate net-like or veil-like membrane or film which usually covers the whole surface in three to four weeks. This often contains warty thickenings. Growth usually remains delicate and viscid so that considerable portions can be lifted up with a platinum needle. In Smith's broth containing 5 per cent. glycerin and 1 per cent. Witte's peptone the bovine type produces alkali so that the original acidity after four to eight weeks lessons or turns to alkali. After about two months the reaction again becomes acid.

The bovine bacillus is usually much more virulent for animals than the human. It will infect guinea-pigs, rabbits, mice, rats, sheep, goats, pigs, cattle, dogs, cats, apes, hedgehogs, canaries, and parrots. The animals die rapidly and from small doses. Thus rabbits succumb from intravenous injections of a thin emulsion from blood-serum cultures in seventeen to twenty-one days (Theobald Smith). The lungs are filled with small gray tubercles and fine nodules are present in spleen, liver, and kidneys. Ravanel[30] produced generalized tuberculosis of rabbits by subcutateous inoculation. Also fatal generalized tuberculosis results from intraperitoneal and intra-ocular injection (Beck[31]). With cattle intravenous injection is fatal in three to four weeks with generalized tuberculosis; also direct injection into lung tissue (Theobald Smith). General infection thus follows intra-ocular (Gaiser), intraperitoneal (Koch and Schütz), intramammary injection (Nocard, Zwick). Calves succumb to feeding with small doses, 10 mg. (Weber and Titze), 1 mg. (English Commission). Here the disease processes are largely in the alimentary canal, affecting the mucus membrane, mesenteric lymph-glands and serous membranes. Caseous pneumonia, more or less acute, follows inhalation of the organisms (Kossel, Weber and Heuss). Subcutaneous



Bovine tubercle bacilli from glycerin-agar culture, fifteen weeks old, stained with carbol-fuchsin, acid alcohol, and methylene-blue.



Turtle tubercle bacilli from ten weeks' culture, stained with carbolfuchsin, acid alcohol, and methylene-blue.

injections of infected material and of pure cultures in calves also results in generalized tuberculosis (Koch and Schütz, 0.05 gr.).

Swine, sheep, goats, cats, and apes are equally sensitive. Less sensitive are dogs, mice, rats, and hedgehogs.

**Tuberculin.**—The toxic substance contained in the proteins of the tubercle bacillus is known as *tuberculin* and various methods have been employed to obtain it. *Old tuberculin* as prepared by Koch[32] was made from tubercle bacilli grown in slightly alkaline 5 per cent. glycerin peptone broth for six to eight weeks. The culture was heated on a water-bath at 80° C. and evaporated to one-tenth its original volume. In consequence of this reduction in volume the final fluid is a 50 per cent. glycerin solution which contains the extracts or products of the tubercle bacillus. The dose of old tuberculin for diagnostic purposes as now usually given is 0.1 to 0.2 milligram. This is prepared by making suitable dilutions of the tuberculin usually in 0.5 per cent. aqueous carbolic acid solution, each cubic centimeter of the original tuberculin being regarded as a gram in weight. *New tuberculin* was prepared by Koch[33] by grinding dried cultures of virulent tubercle bacilli until no intact bacilli were left. One gram of the dried organisms was shaken up in distilled water and centrifugalized. The supernatant fluid naturally contains the water-soluble constituents of the bacillus. It is known as TO (Tuberculin Oberschicht). It has the same physiological properties as old tuberculin. The residue, known as TR (Tuberculin Rüchstand), is dried and again ground up, shaken in water and centrifugalized. This process is repeated several times and eventually all the residue goes into an emulsion. All the emulsions of the residues are mixed. No more than 100 c.c. of water should be used. The amount of solid substance is determined by the evaporation and drying of 5 c.c. quantities. Glycerin water and formaldehyd are added to the residue so that each cubic centimeter contains 0.002 gram of solid material (Ruppel[34]). Among other tuberculin preparations are the *Bacillary emulsion* of Koch,[35] consisting of an emulsion of pulverized tubercle bacilli in distilled water (1 : 100). The supernatant fluid is poured off and 50 per cent. glycerin added as a preservative. Each cubic centimeter contains 5 mg. solid substance. The "filtered bouillon" of Denys[36] is a filtrate from 5 per cent. glycerin peptone broth cultures of the bacillus, 0.25 phenol being added to preserve it. It corresponds to the old tuberculin of Koch unheated. Tuberculin is now used almost entirely for diagnostic purposes. Tuberculous animals and human beings respond to its introduction by a characteristic reaction which consists of fever, general malaise, swelling and tenderness of external lesions.

**Cutaneous Tuberculin Test.**—The reaction known usually as the von Pirquet reaction,[37] follows the cutaneous introduction of old tuberculin, either a 25 per cent. solution or the undiluted material. Drops of the tuberculin are placed upon the skin about 6 cm. apart. The skin is now scarified under these drops and a similar scarification of the normal skin carried out between the drops. The reaction consists of an erythema, small papules and herpetiform vesicles appearing in twenty-four to forty-eight hours.

**Ophthalmotuberculin Reaction.**—This reaction, originally proposed by Wolff-Eisner[38] and Calmette,[39] consists in instilling tuberculin into the conjunctival sac. A sharp congestion of the conjunctiva follows in tuberculous individuals in a few days. Old tuberculin is precipitated with twice its volume of 95 per cent. alcohol, washed with 70 per cent. alcohol, dried between layers of filter-paper and then "in vacuo" over $H_2SO_4$. Solutions of the final product are made in sterile normal salt solution, 1 per cent. by weight, boiled and filtered. These solutions are then diluted to 0.5 or 1 per cent. and a drop instilled into the conjunctival sac. At the present time this test is but seldom employed.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### MYCOBACTERIUM AVIUM Straus and Gamaléia

Discovered by Straus and Gamaléia[1] in tuberculosis of birds. The organisms are very short and slender on glycerin serum, distributed uniformly through the field and not in bunches.

Fig. 5.—Avian tubercle bacillus from ten weeks' culture on glycerin agar, stained with carbolfuchsin.

In old cultures and at high temperatures the cultures tend to form threads and branches (Mafucci). According to Mafucci the avian type produces colonies in eight days on blood-

serum with glycerin which look like small white flecks of wax, punctiform, increasing in extent and thickness. They tend to coalesce and form a white speckled incrustation which is easily lifted from the medium. Older cultures are stringy, viscid, and have a yellow color. In fluid cultures they form a very fine white powder which sticks to the glass and forms a uniform white film on the surface and on the bottom. Strauss[2] finds that the growth on glycerin serum is moist and viscid, drying only after long stay at incubator temperature. The culture masses are soft and delicate and easily broken up on slide or coverslip. The growth is smooth also on various plant media such as potato, carrots, and beets, with and without glycerin, on rice media, on aqueous agar with and without glycerin or grape sugar. They are not crumbly like the mammalian bacillus. In general the avian tubercle bacillus is easier to cultivate and grows more rapidly. It grows at temperatures between 25° and 45° C., the optimum temperature being between 30° and 43° C., and produces more abundant growths. It lives a long time on artificial media, one to two years. Bang[3] reports that avian tubercle bacilli produce a steady decrease in acidity in Theobald Smith broth and finally an alkaline reaction more pronounced than that obtained by Smith from bovine bacilli.

**Pathogenic Action.**—Much more virulent for fowls than mammalian tubercle. Intravenous injection of small quantities produces emaciation and death in a short time. Intraperitoneal and intramuscular injection also fatal, the latter probably by toxemia. Infection also easily produced by the feeding of cultures and of infected material. Other birds which can be infected by feeding are hawks, sparrow-hawks, canaries, parrots, pigeons, and ducks. Mice are also sensitive especially to intraperitoneal injection (Roemer) and also to feeding and subcutaneous injection.

Rabbits are usually sensitive, guinea-pigs somewhat so. Cattle in general are resistant to the avian bacillus although infection from subcutaneous injection does occasionally occur. Goats die from intravenous inoculation. Dogs and horses are generally resistant.

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### Tuberculosis of Cold-blooded Animals

Two types of tuberculosis occur in cold-blooded animals, the tuberculosis which occurs as the direct result of injections of mammalian and avian tubercle bacilli on the one hand and spontaneous tuberculosis due to a quite independent group of acid-fast organisms on the other hand. In the latter groups spontaneous tuberculosis has been observed in serpents, carps, turtles, and frogs. Weber and Taute[1] believe that the bacilli producing spontaneous tuberculosis of this character are acid-fast saprophytes from moss, earth, and mud which frequently get into the bodies of cold-blooded animals and produce local or general disease processes.

### Mycobacterium tropidonatum (Sibley)

First observed by Sibley[2] in a reptile, *Tripodonotus natrix* var. *murorum*, which after several months in captivity developed three tumors the size of a hazelnut on the side of the body, and small isolated nodules in the connective tissue of the peritoneal cavity and spinal column, in the kidney and at the bifurcation of the aorta. The liver and spleen were filled with nodules the size of a pea. All the nodules contained caseous material which consisted microscopically of round cells and masses of acid-fast bacteria. No epithelioid cells or giant-cells. The acid-fast organisms looked and stained like ordinary tubercle bacilli and Sibley regarded the disease as true tuberculosis. No cultures.

Hanseman[3] found in the peritoneal cavity of a *Pyphon reticulatus* (Riesenschlange) in the neighborhood of the pancreas a bunch-like body made up of nodules the size of a pea. These contained granulation tissue with round cells, no caseation and no giant-cells, and showed acid-fast bacilli arranged like leprosy bacilli in cells. No cultures.

### MYCOBACTERIUM PISCIUM (Dubard and Terre)

Described by Bataillon, Dubard, and Terre[4] as *Bacillus tuberculosis piscium* in a carp which had a tumor the size of a pigeon's egg next to the ovary. This tumor contained a soft, not caseous, easily divided mass, which on staining by Koch-Ehrlich's method showed great numbers of acid-fast bacilli like tubercle bacilli. On section the tumor showed giant-cells, epithelioid cells and round cells. The acid-fast organism was cultivated at room temperature, growing from 12° to 36° C. Optimum at 25° C. Strongly aërobic. No growth in gelatin or agar stab. Broth showed abundant development in two to three days, the broth remaining clear, a delicate fragile scum appearing on the surface, with a flocculent easily separated sediment. On potato white warty colonies of soapy consistency. On agar round, white, creamy colonies in three to four days, occasionally confluent. The agar growth after about eight days showed no typical bacilli but only interlacing

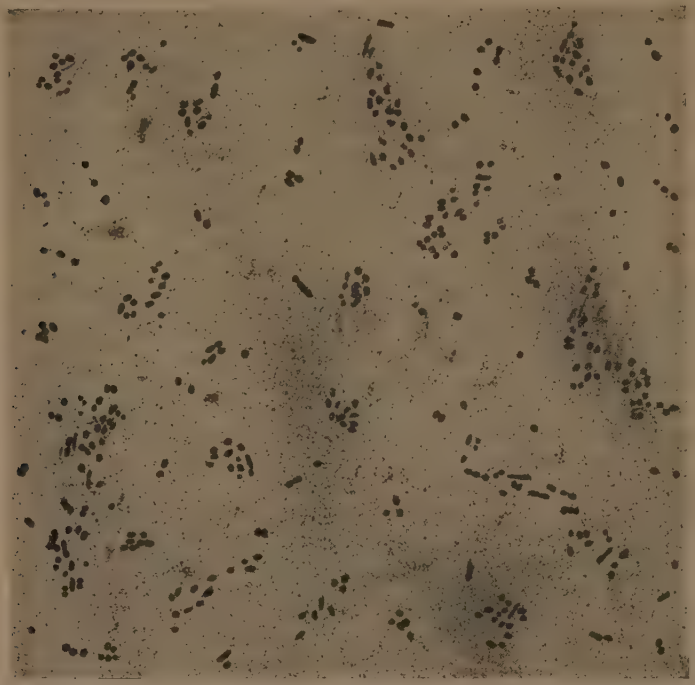

Fig. 6.—Fish tubercle bacilli from sixteen-day culture on glycerin agar, stained with carbol-fuchsin, decolorized with 10 per cent. $H_2SO_4$, counterstained with methylene-blue.

masses of bacteria with branching and pointed end-processes. Stained by Ehrlich's method the ground masses were blue and contained many sharply stained red granules. Growth in gelatin very slow, colonies appearing only after three weeks, dry with opaque centers, peripheral zone more transparent and jagged. No liquefaction. Growth at 37° C. scanty, resembling that of human tubercle bacilli. Acid-fastness the same as that of true tubercle bacilli, the organisms not being decolorized by hot alcoholic hydrates. Kräl and Dubard[5] report that the fish tubercle bacilli exhibit greatest acid-fastness when cultivated on glycerin sugar and peptone media but less or even none when transferred to media poor in nitrogen. The bacilli develop into threads which show swelling of the protoplasm from which side branches

develop. By frequently shaking broth cultures a homogeneous development occurs with turbidity. Hanging drops show motility. Sterile milk becomes thick but does not coagulate. Cultures at 37° C. not to be differentiated from those of human tubercle bacilli except by microscopic appearances. Carp are resistant to artificial infection. Frogs succumb in ten to fifteen days, the lungs and liver showing tuberculosis only occasionally. Lizards are also sensitive to intraperitoneal inoculation, dying in eight days if kept at ordinary temperatures and in about a month at 37° C. Pigeons and guinea-pigs not sensitive.

It was later shown by Dubard[6] that *Bacillus tuberculosis piscium* could be accustomed to 34° C., at which temperature it grew slowly and produced a brownish-black pigment. Old broth cultures had an odor of sprouting vegetables, cultures on solid media, the typical odor of tubercle bacilli. Organisms both acid-fast and non-acid-fast.

### MYCOBACTERIUM CHELONEI (Friedmann)

Observed by Friedmann[7] in a turtle (Schildkrote) three months in confinement and fed by a tuberculous attendant. The right lung was infiltrated with caseous nodules, with an abscess cavity about 10 cm. in diameter. Acid-fast bacilli were found in the bronchial mucous and in the diseased

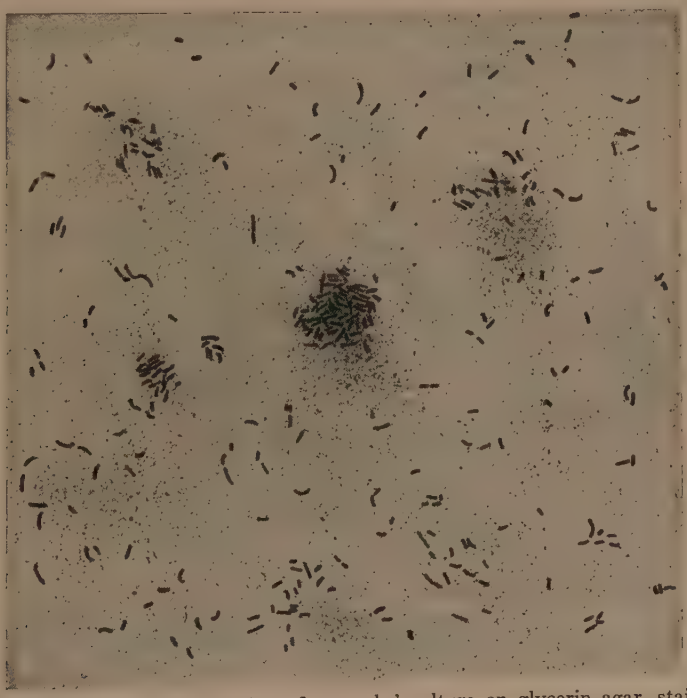

Fig. 7.—Tubercle bacilli from the frog, five weeks' culture on glycerin agar, stained with carbolfuchsin and methylene-blue.

tissues which also showed round cells and giant-cells. Obtained in pure cultures. On glycerin serum a fine bluish veil appeared in four days at 25° C. isolated dry colonies in seven days and an expansion over the entire surface of the media in three weeks. On glycerin gelatin radiating bands occurred in eight days, with confluent nodules. No liquefaction. Growth slower at

37° C. as a finely granular, grayish-yellow film or skin which gradually became powdery, showed wrinkles and folds and resembled mammalian tubercle bacilli. In young agar cultures acid-fast and non-acid-fast organisms were found.

### MYCOBACTERIUM RANÆ (Ruprecht and Küster)

Spontaneous tuberculosis in frogs was first recognized by Küster[8] in 3 frogs out of 200 examined in the vicinity of Freiburg. The first report on it was published by Ruprecht.[9] The animals showed multiple nodules in the liver, the size of a pea, filled with yellowish-white cheesy material. Microscopic examination revealed leukocytes and homogeneously stained acid-fast bacilli but not giant-cells.

Pure cultures of an acid-fast organism were obtained in agar and on serum. Growth aërobic. Optimum temperature 25° C. No growth above 25° C.

**Morphology.**—Non-motile organisms stained by ordinary anilin dyes and by Gram. Acid-fast even when stained cold. Young cultures show some organisms which are not acid-fast while septate involution forms with granular staining are found in old cultures.

**Cultural Characters.**—Development is visible on agar and serum in four days, that on serum resembling the growth of the avian tubercle bacillus. The colonies are separate, nail-head, whitish-gray, glistening, soft, and easily raised. When transferred to a drop of water on a coverslip the material spreads like oil. On dry agar scaly elevations appear at times, like the growth of the human tubercle bacillus. The addition of glucose to media favors development. Abundant multiplication in milk, which is first thin and fluid, then peptonized, finally clear yellow and alkaline. Broth shows occasionally a diffuse turbidity, usually a cloudy sediment. When transfers are very carefully made a dry, clefted, superficial film appears. No liquefaction of gelatin.

Pathogenic for all cold-blooded animals, frogs, lizards (*Lacerta agilis*), turtles, serpents (*Ringelattern*), slow-worms, crabs, snails, salamanders, and water salamanders. Also pathogenic for mice, rabbits, and guinea-pigs in large doses (toxins?) and occasionally for rats.

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### BACILLUS LEPRÆ Hansen

Observed originally by Hansen[1] in nodules from leprosy cases, his discovery being duplicated the same year by Neisser.[2] Acid-fast and closely related to the tubercle bacillus with which it is grouped in the mycobacteria (Jadassohn[3]).

**Morphology.**—Very fine rods about 0.2 to 0.45 $\mu$ wide and 1.5 to 6 $\mu$ long. Longer in fresh nodules than in old, in the skin than in the internal organs. Usually straight but may be bent or curved. Occasionally pointed on the ends. Difficult to see in tissues when unstained. According to Babes,[4] leprosy bacilli show short branches which end in club-shaped or pear-shaped extremities. These are regarded by Jadassohn as involution processes. The organisms tend to appear in masses, in bunches like cigars, and are more frequently intracellular than tubercle bacilli. Non-motile. No spores. No capsules.

**Staining Reactions.**—Acid-fast, staining by all the methods applicable to the tubercle bacillus, Ziehl's, Ehrlich's, Koch's original, etc. Jadassohn states that leprosy bacilli take up dyes more rapidly than tubercle bacilli and are more easily stained by aqueous or aqueous-alcoholic solutions of anilin dyes. This however is not of diagnostic value. Some organisms in preparations stain homogeneously, some are definitely granular. This is especially true of old individuals. With strong acids part of the organism may decolorize and take the counterstain. Leprosy bacilli frequently show the presence of Babes-Ernst or metachromatic

granules, when stained by methylene-blue, Bismarck brown, methylene-blue in acetic acid, etc. Stained by Neisser they show small unstained spheres or vacuoles of various form, terminal or central, which may resemble spores. Such individuals look like diplococci. Gram positive. Stained with osmic acid, the organism shows black granules of fat or wax like those in tubercle bacilli. Granules such as Much has described in the tubercle bacilli may also be demonstrated in the organisms of leprosy.

When the organisms appear in masses, a homogeneous substance with the same staining properties is found in their neighborhood. This has been described by Unna as "Bacillenschleim" but later interpreted as dead bacilli.

**Distribution.**—The leprosy bacillus is present in all the lesions of the skin, in the so-called lepromata, in the internal organs, liver or spleen, of advanced cases, in the sputum when the lungs are infected, in the nasal secretions, etc.

It is found in the lesions in close association with tissue elements which show a characteristic pathological-histological reaction. It lies in the tissue spaces but is especially characterized by its tendency to be found inside cells.

In smear preparations from leprous material, secretions of the nose and bronchi, etc., leprosy bacilli may be demonstrated by the usual staining methods. In the sputum it is best demonstrated by the antiformin method of Uhlenhuth and Steffenhagen for the demonstration of tubercle bacilli. The diagnosis of leprosy has to be established often however by the demonstration of leprosy bacilli in the tissues after fixation and hardening. For this purpose celloidin preparations are not valuable and fixation in alcohol or in Müller's fluid gives best results. The methods suggested by Darier[5] (modification of Ziehl), Fick,[6] Gurd,[7] and Unna[8] may be recommended.

**Differentiation from Tubercle Bacilli.**—Various attempts have been made to differentiate leprosy bacilli from tubercle bacilli. In general leprosy bacilli are somewhat more easy to stain and to decolorize than are the organisms of tuberculosis. Furthermore the leprosy bacilli seem to vary more in acid-fastness than do tubercle bacilli. All such distinctions are quantitative however and at present no definite criteria of differentiation have been established. Baumgarten[9] claims that treatment with dilute fuchsin solution twelve to fifteen minutes, followed by alcoholic nitric acid (1 : 10) and counterstaining with methylene-blue, stains leprosy bacilli but not tubercle bacilli. Babes finds leprosy bacilli can be stained by his anilin-safranin-iodin-method but not tubercle bacilli. Another method which is thought to give differential results is that of Yamamoto[10] who stains ten minutes in warm 5 per cent. silver nitrate, five minutes in 2 per cent. pyrogallol and 10 per cent. tannin for reduction of the silver. This author states that tubercle bacilli appear black, leprosy bacilli light and unstained.

**Transmission to Animals.**—Frequent efforts have been made to transmit leprosy to all types of the lower animals and almost without exception these attempts have met with failure. Thus Hansen attempted to infect rabbits, cats, and apes with material containing leprosy bacilli in large numbers but without success, and Neisser made similar experiments with rabbits. In dogs Neisser produced by subcutaneous injection local areas of inflammation which resembled the lesions in human leprosy. Such local "lepromata" contain a great many leprosy bacilli at first, later a few and finally none can be demonstrated. The tissue reaction is usually explained as a foreign-body reaction. Somewhat more suggestive results follow intra-ocular injection. Thus among others Damsch[11] introduced the organisms into the anterior chamber of the eye of rabbits and found changes in the cornea, Desmet's membrane and in the anterior capsule of the lens. Some of the cells contained leprosy bacilli and similar cells with bacilli were present in the iris and the ciliary body. Damsch also found granulation tissue in the neighborhood of leprosy bacilli introduced subcutaneously and intraperitoneally in cats.

Especially interesting are the results of Melcher and Ortmann[12] who endeavored to produce "metastatic leprosy" by repeated intra-ocular injections in rabbits. After treatment extending over several months disseminated nodules (?) were found in the lungs, intestines, and lymphatic glands, which showed considerable resemblance to both leprosy and tuberculosis. These lesions are not usually accepted as definitely leprous however.

Finally Sugai[13] injected various animals intravenously and intraperitoneally with emulsions of large fresh leprosy nodules. The most suggestive results were obtained with Japanese dancing mice which sometimes sickened and died in three to four weeks. In these animals, and in similar mice which remained well and were killed, larger or smaller granulomata (up to the size of peas) were found in the peritoneal cavity. The nodules showed caseous centers, round cells, and epithelioid cells. Somewhat later Duval[14] inoculated rats, white mice, and Japanese dancing mice with material from a case of tuberculous leprosy. The white mice died in fourteen days and many whitish-gray nodules, closely resembling leprosy nodules, were present in the peritoneal cavity.

Local lepromata have also been produced in apes by Nicolle.[15] These were at first absorbed, but following repeated injections larger nodules developed which were much slower to absorb. Somewhat similar results were obtained by Marchoux and Bourret[16] in chimpanzees.

In general, leprosy cannot be said to have been produced experimentally in animals by the use of material directly from human cases.

Leprosy bacilli injected into animals survive for long periods. Thus Leloir[17] claims to have demonstrated them two and one-half years after intraperitoneal injection. Imanow[18] also, after subcutaneous and intraperitoneal injection of emulsions of skin lepromas, found that the bacilli were rapidly taken up by phagocytes and after eight months were still demonstrable in large numbers.

In all attempts to transmit leprosy to lower animals like rats especial precautions must be taken to avoid mistaking the lesions for true leprosy when in reality they are the lesions of an independent disease, rat leprosy.

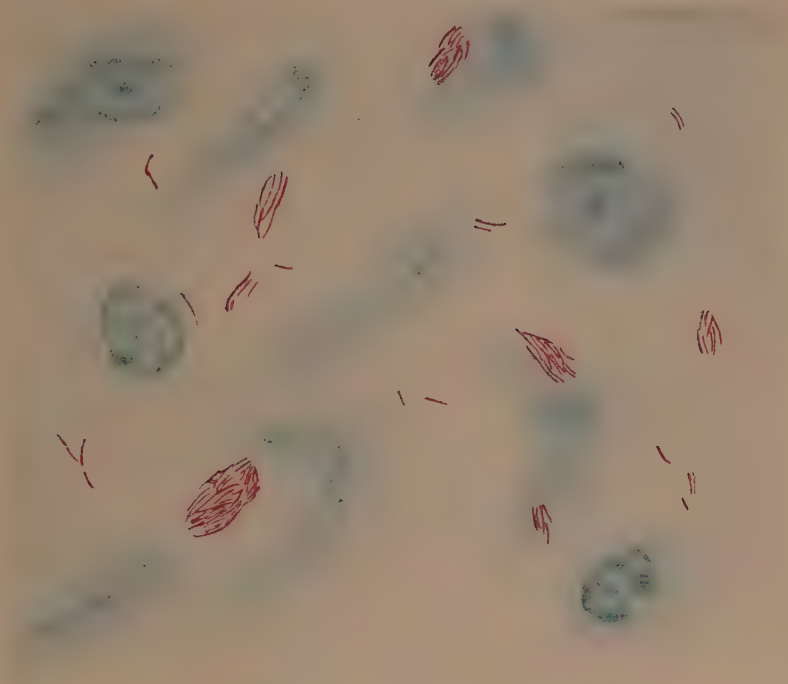
**Cultivation.**—Since its discovery by Hansen many different attempts have been made to cultivate the leprosy bacillus and from time to time acid-fast organisms have been obtained in cultures which resemble the leprosy bacilli found in the tissues. It is impossible to say that any of the cultivated acid-fast organisms are really leprosy bacilli because thus far no animals have been found which are susceptible to leprosy and none of the acid-fast strains produce lesions in animals which are in any way comparable to the lesions of leprosy in man. It may be said in passing that since animals do not acquire leprosy the failure to produce lesions may not be a sure proof that some of the acid-fast strains are not true leprosy bacilli. A few only of the reputed successful cultivations need be mentioned. Kedrowsky[19] attempted to cultivate leprosy bacilli on aqueous extracts of placenta and agar and on serum-nutrose-agar and obtained organisms which he placed in the streptothrices or actinomyces along with the tubercle bacillus and the diphtheria bacilus. He believed that the rod-shaped leprosy bacilli were only a stage in the life cycle of an organism resembling actinomyces. From the leprosy nodules (leproma) acid-fast bacilli, or diphtheroids with acid-fast granules, grew rarely branched threads. Injected into the lymphatic system of rabbits these organisms produced lesions like tuberculosis, into the blood-stream, small nodules with countless bacilli. Cultures from such lesions showed both acid-fast and acid-sensitive organisms.

Similar organisms belonging to the streptothrices or actinomyces were obtained by Williams.[20] Some of the elements were acid-fast, some acid-sensitive, some diphtheroids. The acid-fast bacillary forms injected in large quantities into guinea-pigs produced leproma-like nodules with many bacilli in the connective tissue.

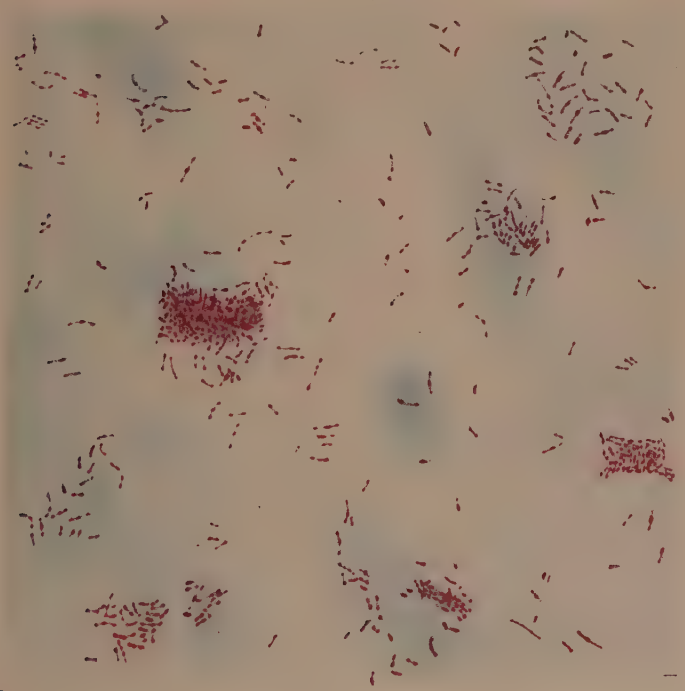
More successful results were obtained by Clegg[21] who used alkaline meat-infusion-agar and inoculated it with amebæ and symbiotic bacteria from dysentery stools. After these were grown the cultures were again inoculated with leprosy bacilli from lepromas from the ear and from the spleen. After six days in the thermostat, short plump acid-fast bacilli could be seen along with the leprosy bacilli. These acid fast bacilli could be cultivated on the same medium up to the tenth generation. The method of Clegg was further amplified by Currie, Brinckerhoff, and Hollmann.[22] The amebæ obtained from the intestines of guinea-pigs were cultivated with cholera spirilla for five days at 37° C. till the amebæ were encysted. This material was then inoculated with leprosy bacilli from non-ulcerated lepromas. Acid-fast organisms grew and subsequently, by heating to 60° C., were cultivated on ordinary media where they produced an orange or brownish-yellow color. In guinea-pigs they produced a diffuse thickening and loss of hair at the site of subcutaneous inoculation. No lesions developed in white rats or monkeys. Duval[14] and Duval and Gurd[23] cultivated acid-fast bacilli from leprosy material in symbiosis with other bacteria and on media containing amino-acids (tryptophan and cystin) (bananas with 2 per cent. tryptophan or cystin). After about fourteen days long thin acid-fast bacilli with metachromatic granules developed and could be transferred to the same medium, to glycerin agar with juice from turtle meat, and to Novy and McNeal's agar with glycerin. In fluid cultures these organisms grew on the surface, first colorless then citron or orange-yellow. They were pathogenic to guinea-pigs and to Japanese dancing and white mice. The latter showed small circumscribed non-caseating nodules. Lesions like leprosy were also produced in apes (*Macacus rhesus*).

Finally partially acid-fast diphtheroids have been cultivated from leprosy by Wolbach and Honeij.[24]

**Non-chromogenic Acid-fast Organisms.**—Organisms of this type were first obtained from leprosy by Weil, subsequently by Karlinski, by Marchoux and by Twort. A complete study of them was made by Duval. The organisms were cultivated in trypsinized human serum, egg albumin, liver or placenta tissue, or in similar tissues autolized at 42° C. for two weeks, or hydrolized by Finkler-Prior vibrio twenty-four to thirty-six hours at 37° C. The filtrates from these products are added to 4 per cent. agar giving "amino-acid agar." Cultures were obtained by Duval from 8 cases. Colonies were pin-head, elevated, colorless, translucent, easily removed from the medium and forming a uniform turbidity in broth. Organisms longer and thicker than bacilli in leprosy tissues, varying from solidly staining short and long rods with pointed or swollen ends to irregularly staining long and short, straight and slightly curved rods containing isolated spherical masses of chromatin. Other forms are spindle-shaped bacilli with greatly swollen centers, long forms, often dumb-bell and club-shaped. No branching or filamentous form. Non-motile. No spores. Completely acid-fast as compared with the tubercle bacillus. Non-pathogenic.



Leprosy bacilli film from a nodule, stained by Ziehl-Neelsen.



Acid-fast bacilli cultivated from leprosy nodules, film from fourteen-day culture on glycerin agar, stained with carbolfuchsin and methylene-blue.

**Anaërobes in Leprosy.**—Anaërobes were first isolated from leprosy by Ducrey[25] from a nodule in the face in a case of tubercular anesthetic leprosy. They were cultivated anaërobically in broth, agar, 5 to 10 per cent. glycerin agar, solidified beef serum, solidified hydrocele fluid and 3 per cent. glucose agar. Growth along the line of puncture occurred in glucose agar as a delicate line of minute colonies. After fifteen days a nebulous zone of minute colonies extended into the medium, 2 to 3 cm. below the surface. Growth in ordinary agar, glycerin agar, blood-serum, and glycerin broth only when the tubes were put in a vacuum. The broth remained clear, had a granular deposit at the bottom and a white surface pellicle. No growth on potato or in gelatin. Old glucose agar cultures kept one year at 20° C. gave only anaërobic transfers. The organisms resisted decolorization by the Koch-Ehrlich method but were decolorized by Ziehl-Neelsen and Gabbet. Non-pathogenic.

Subsequently Campana[26] obtained a small homogeneous bacillus staining irregularly and showing bars, granules and swollen segments at one or both extremities and in the middle, the latter type sometimes club-shaped. Slightly acid-fast, decolorizing by the Koch-Ehrlich method and dilute alcohol. Cultivated under anaërobic conditions in glucose agar at 37° to 40° C. Non-pathogenic.

An organism with practically the same reactions was obtained by Serra.[27]

From this review it is evident that four different types of organisms have been cultivated from leprosy in different parts of the world, acid-fast organisms tending to form long threads and branches, acid-fast chromogenic bacilli forming a yellow pigment on media, non-chromogenic acid-fast bacilli and partially acid-fast diphtheroids. As representative of these various types the strains of Clegg, Duval, and Wolbach and Honeij may be briefly considered.

### Clegg's acid-fast organism from leprosy

Isolated on nutrient agar by the introduction of leprosy material into a culture of amebæ and cholera vibrios. Acid-fast organisms begin to multiply in about a week. By heating to 60° C. half an hour the amebæ and cholera vibrios are killed and the acid-fast organisms survive. Pure cultures of acid-fast bacteria may now be obtained.

**Morphology.**—Non-motile bacilli from 1 to 5 $\mu$ in length. Young forms straight or curved. Long slender forms occur. No branching. No spores.

**Staining Reactions.**—By Neelsen's method the short forms and many of the long forms stain uniformly. In many instances certain portions of the bacilli stain more deeply than others. In young cultures the centers stain more deeply than the poles. Some organisms are beaded showing alternating deep and lightly stained areas. Stained faintly by ordinary alkaline dyes. Young cultures retain the stain when treated with hot carbolfuchsin and Gabbet's solution. Resist decolorization three minutes in absolute alcohol. Many of the organisms lose the stain on treatment two minutes with 5 per cent. HCl in alcohol. Others are not decolorized. Old cultures resist decolorization less than young cultures but resist Gabbet's solution and absolute alcohol.

*Glycerin Agar.*—Growth from canary-yellow to deep orange, slimy or granular, spreading slightly from line of inoculation. Surface smooth and shining or rough and nodular, never truly wrinkled. Edges of growth smooth, raised, sharply defined. Growth always moist. On neutral media growth drier and more granular, on fully alkaline media growth more moist and even slimy.

*Plain Agar.*—Growth thinner, spreads little from line of inoculation and has the same general character as that on glycerin agar.

*Nutrient Agar.*—Growth heavier than on plain agar, not so heavy as on glycerin agar, light yellow and reddish-yellow (orange), limited to line of inoculation. Surface smooth but may be nodular from heaping up of the colonies.

*Dorset's Egg Medium.*—Growth invariably deep orange-yellow, never lighter yellow. Growth spreads little, very dry, mealy, granular, not wrinkled or glistening. Borders clearly defined, slightly raised. No liquefaction at the end of a month.

*Loeffler's Serum.*—Spreading growth, light orange-yellow to canary-yellow. Surface moist, smooth, and glistening. Growth slimy and tenacious. No liquefaction.

*Gelatin Stab.*—Heaped-up growth at point of puncture with very slight growth along line of inoculation. Growth heavier in upper part of line than below. No liquefaction.

*Potato* (neutral).—Growth limited to line of inoculation, deep orange-yellow, dry and mealy. Surface rough from the coalescence of numerous small colonies, not wrinkled.

*Broth.*—Fine flocculent growth adhering to the sides of the tube from which it sends out stalactites. No turbidity. No pellicle. Thick tenacious sediment. In old cultures a heavy wrinkled scum develops turning golden-yellow.

*Glycerin Broth.*—More abundant growth of the same character.

*Dunham's Peptone.*—Yellowish sediment sending up flocculent threads which float in the media. Fluid clear. No pellicle.

*Glucose broth* in fermentation tubes. Heavy, slightly wrinkled, yellow pellicle in open bulb adherent to the walls of the tube. No growth in closed arm. No gas.

Lactose, mannite, and saccharose broth the same.

**Pathogenic Action.**—Pure cultures produce lesions in guinea-pigs, not in white rats and monkeys. Lesions after subcutaneous inoculation consist of diffuse thickening and loss of hair. Microscopically the lesions consist of richly cellular granulation tissue made up almost exclusively of irregular spindle-shaped young connective tissue cells. The cells contain numerous nuclei. Giant-cells present. Bacilli numerous in lesions. Horses treated with live cultures produce an agglutinating serum, active in a dilution of 1 : 1000.

### DUVAL'S ACID-FAST CHROMOGENE

Cultures obtained by Clegg's method on bananas to which 1 per cent. solutions of tryptophan, cystin and leucin were added and on 2 per cent. nutrient agar with the same substances. Optimum temperature 32° C. Growth better in the light than in the dark. Cultures obtained from 4 cases as semitranslucent grayish-white colonies, later becoming orange-yellow. Also cultivated on coagulated egg-albumin and on blood-serum bathed in 1 per cent. sterile solution of trypsin. After a few generations transfers can be made to glycerin serum agar to which an infusion of turtle muscle is added. Cultures finally made on ordinary blood agar and ordinary agar.

In cultures the organisms are arranged in masses of parallel bacilli like the leprosy bacilli in tissues. They are straight or curved and stain irregularly. They vary greatly in length, some being short and thick, almost coccoid, others long and slender. They contain metachromatic granules and alternating light and dark stained areas. Long filamentous bacilli occur with blunt and swollen ends, shorter forms with pointed ends.

The bacilli are acid-fast like leprosy bacilli, less acid-fast than tubercle bacilli. They resist decolorization with Gabbet's solution, weak acids and alcohol. They are slowly decolorized by 30 per cent. nitric acid followed by 95 per cent. alcohol, not sufficient to decolorize the tubercle bacillus.

Lesions produced in Japanese waltzing mice like those in leprosy. Disseminated infection produced in monkeys (*Macacus rhesus*) with lesions like those in human leprosy.

### WOLBACH'S PARTIALLY ACID-FAST DIPHTHEROID

Obtained by Wolbach and Honeij from an epitrochlear lymph-node from a case of tuberculous leprosy. According to the authors this culture corresponds in all respects to the majority of diphtheroids isolated from leprosy in various parts of the world.

**Morphology.**—Extremely pleomorphic, the morphology varying with the medium employed and with the age of the culture. All cultures show acid-resistant granules and segments and non-acid-resistant short forms, usually in pairs which resemble cocci in appearance. The latter are particularly numerous in young cultures. Other non-acid-resistant forms are slender paired bacilli which usually have a parallel arrangement and resemble closely the bacillus of diphtheria after twelve to sixteen hours' cultivation on Loeffler's blood-serum. The smallest bacilli containing acid-resistant granules are 1.5 to 2 $\mu$ in length and from 0.2 to 0.3 $\mu$ in width. A characteristic appearance in young cultures is that of bacilli with bipolar acid-resistant granules although a single bacillus may contain three or more acid-fast granules or segments. After a few days incubation long swollen bacilli, resembling the involution forms of diphtheria bacilli, make their appearance. In these forms the swollen ends are usually acid-resistant. They contain in addition acid-resistant granules and transverse bars. Occasionally slender bacilli are completely acid-resistant when treated by the methods described below. The large diphtheroid involution forms reach the dimensions of 8 to 10 $\mu$ by 0.2 to 0.6 $\mu$. All the forms described above may be found in the same culture, after one to two weeks incubation upon favorable media. Acid-resistant bacilli are more common in young cultures than in old. Non-motile.

**Staining Reactions.**—The bacilli are strongly Gram-positive and stain by the ordinary anilin dyes, though less intensely than most other bacteria, and markedly less than the diphtheria bacillus. After staining with carbolfuchsin, they resist decolorization to the extent that portions of the bacilli remain a bright red after treatment with 1 per cent. HCl in 70 per cent. alcohol, 2 per cent. $H_2SO_4$ followed by 95 per cent. alcohol and 3 per cent. HCl in 95 per cent. alcohol. Twenty per cent. sulphuric acid leaves the granules with a faint reddish tinge, although bacilli from litmus milk are left distinctly red after staining by

Gabbet's method for the tubercle bacillus. The films were treated with the decolorizing reagents for thirty seconds, similar treatment completely decolorizing the diphtheria bacillus, staphylococci and mixed cultures of bacilli and micrococci such as are found in the cultures from the throat.

**Cultural Reactions.**—Isolated in aërobic ascitic fluid dextrose agar by the introduction of a bit of the excised gland. After about ten days a translucent whitish band appeared around the piece of gland tissue. Transfers gave a poor growth in broth with or without glycerin, and on plain agar, dextrose agar, and glycerin agar. After several months cultivation the bacillus grew readily on these media. No growth on potato with or without the addition of 0.5 to 1 per cent. sodium carbonate. Glycerin does not favor the growth.

Growth at 37° C. No growth at 18° to 21° C.

After several months, cultivation at 37° C. the colonies are the same on all solid media. The rate of growth is more rapid on agar media containing ascitic fluid or brain tissue. On *agar media* the colonies become visible in forty-eight hours. They have at first a moist appearance and are colorless and transparent. At the end of a week they reach a diameter of 0.5 to 1 mm. and become grayish-white in color. After prolonged cultivation the colonies become confluent and develop their characteristic appearances. The color is grayish-white and translucent. The surface is rather dry and granular in appearance. The colonies do not become markedly elevated although a characteristic feature is a central elevation. The edges of the colonies are irregular. In discrete colonies the edges are serrated or auricular. Where the growth extends from confluent colonies the extensions are arborescent and new colonies frequently take origin at the borders of older ones. Under low magnification they are seen to be coarsely granular in structure. On *egg media* the colonies have a distinct yellowish cast. On *Loeffler's blood-serum* and *Bordoni-Uffreduzzi's medium* they are more opaque and white in appearance with a suggestion of yellow. Growth on *brain agar* is similar to that on serum-containing media. In stab cultures in *plain* or *dextrose agar* growth takes place for a depth of 4 cm. The colonies remain discrete when examined with the hand-lens and there is a rather characteristic grouping of the colonies which extend into the medium from the line of puncture because of a tendency to cluster formation, produced by the origin of new colonies at the peripheries of old ones. *Broth* with or without the addition of dextrose and glycerin remains clear. Growth takes place along the sides of the tubes and at the bottom. No pellicle formation. *Litmus milk* not changed in appearance.

Acid production in dextrose, maltose, and glycerin but not in lactose, saccharose, raffinose, mannite, or inulin.

Old cultures have a peculiar fruit-like odor which is not unlike that of the bacillus of tuberculosis.

Killed in ten minutes at a temperature of 60° C. A few organisms survive 60° C. applied for five minutes.

**Non-pathogenic** for Japanese waltzing mice, guinea-pigs, rabbits, and white rats. The rabbits were inoculated intravenously, subcutaneously, and intraperitoneally, the mice, guinea-pigs, and rats subcutaneously and intraperitoneally.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   ———: Virchow's Archiv., 1881, lxxxiv, 514.
3. Jodassohn: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, v, 791.
4. Babes: Nothnagel's Handbuch. Article on Leprosy.
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6. Fick: Arch. f. Dermat. u. Syph., 1908, xcii, 409.
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7. Gurd: J. Infect. Dis., 1911, viii, 39.
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9. Baumgarten: Monatsch. f. prakt. Dermat., 1884, iii, 193.
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12. Melcher and Ortmann: Berl. klin. Wchnschr., 1885, xxii, 193. *Ibid.*, 1886, xxiii, 135.
13. Sugai: Japan Zeit. f. Derm., 1911, ii, Hft. 2.
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16. Marchoux and Bourret: Ann. de l'Inst., Pasteur, 1909, xxiii, 513.
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    ———: Ann. de dermat., 2. s., 1887, viii, 625.
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19. Kedrowsky: Ztschr. f. Hyg., 1901, xxxvii, 52.
20. Williams: Brit. M. J., 1911, ii, 1582.
21. Clegg: Philippine J. Sc., s. B., 1909, iv, 77, 403.
22. Currie, Brinckerhoff and Hollmann: Pub. Health Rep. U. S. Mar. Hosp. Serv., 1910, xxv, 1173.

23. Duval and Gurd: Monatsh. f. prakt. Dermat., liii, 23.
———: J. Cutan. Dis., 1911, xxix, 274.
24. Wolbach and Honeij: J. Med. Research, 1914, xxx (n. s. xxv), 1.
25. Ducrey: Gior. ital. d. mal. ven., 1892.
26. Campana: Clin. dermosifilopat. d. r. Univ. di Roma, 1894, Fasc. 1.
27. Serra: Ges. f. Dermat. u. Syph., 1909.
———: Gior. ital. d. mal. ven., 1910, li, 312.

For a complete review of the various attempts to cultivate the leprosy bacillus *see* Wolbach and Honeij, J. Med. Research, 1913–14, xxix (n. s. xxiv), 367.

### Bacillus of rat leprosy

First observed by Stefansky[1] in rats (*Mus decumanus*) in Odessa, 4 to 5 per cent. of which were infected. Reported also in London by Dean,[2] in New South Wales by Tidswell,[3] in Berlin by Rabinovitsch,[4] and in this country in California by Wherry[5] and by McCoy.[6] The disease presents a curious agglomeration of lesions. The lymphatic glands may be enlarged and hard with areas of softening and the body covered with larger or smaller whitish areas in which the hair is either very scanty or lost and in which nodules, sometimes ulcerating, appear. The skin appears thinned, with an absence of fat tissue beneath, while the muscles are grayish-white, soft, and friable. The internal organs are seldom changed, but an acute form of the disease may occur with bronchopneumonia and septicemia (Currie and Hollmann[7]).

In rat leprosy peculiar acid-fast bacilli are found in the changed muscular tissue, in the lymphatic glands, and in the internal organs like the liver and spleen, as well as in the nasal mucosa. They are frequently intracellular, 3 to 5 $\mu$ long with rounded or slightly bent ends. They stain with cold carbolfuchsin in fifteen minutes, in hot carbolfuchsin in a few seconds and resist decolorization by 5 per cent. sulphuric acid (Stefansky) and 25 per cent. sulphuric acid (Dean). Kitasato[8] reports that they are decolorized in 3 per cent. hydrochloric acid alcohol somewhat more rapidly than leprosy bacilli. Wherry states that they are not decolorized in 5 per cent. nitric acid but are decolorized in 20 per cent. nitric acid in alcohol. Gram-positive. Often granular.

The disease is transmissible from rat to rat by the use of infected material but not to mice, guinea-pigs, rabbits, or apes. According to Wherry and McCoy it is probably conveyed naturally by fleas.

Attempts at cultivation have not thus far been very successful. Zinsser and Cary[9] found that the organisms multiplied in plasma preparations of growing rat spleen. Chapin has succeeded in cultivating them on trypsin-egg-albumin. Zinsser and Cary state that the organisms of rat leprosy retain their acid-fastness in leukocytes but degenerate rapidly inside spleen cells.

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2. Dean: Centralbl. f. Bakteriol. [etc.], 1903, xxxiv, 222.
———: J. Hyg., 1905, v, 99.
3. Tidswell: *See* Brinckerhoff, Pub. Health Bull., 30, 1910, Part 4.
4. Rabinovitsch: Centralbl. f. Bakteriol. [etc.], 1903, xxxiii, 577.
5. Wherry: J. Am. M. Ass., 1908, l, 1903.
6. McCoy: Pub. Health Rep., xxiii, 981.
7. Currie and Hollmann: Pub. Health Bull., No. 41, 1910, p. 13.
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### Mycobacterium paratuberculosis Johne and Frothingham

Found by Johne and Frothingham[1] in a chronic disease of the intestinal tract of cows resembling tuberculosis. The mucus membrane of the intestine contains small nodules made up of lymph-follicles containing leukocytes, epithelioid cells, giant-cells, and small slender bacilli. These bacilli are a little like mammalian tubercle bacilli and are acid-fast by Ziehl's carbolfuchsin followed by Gabbet's solution and by Koch-Ehrlich's stain followed by nitric acid, sulphuric acid, or alcohol applied one-half to fifteen minutes.

The disease is known as Johne's disease, Lolland's disease, pseudo-infectious intestinal catarrh, "enteritis chronica bovis," or paratuberculosis. It is apparently widely spread, having been reported from England, South Africa, Denmark, and America. The organism found in the disease is also called *Bacillus paratuberculosis*, *Bacillus* or *Mycobacterium enteritis pseudo-tuberculosis bovis*, and more frequently Johne's bacillus.

**Cultivation.**—Johne's bacillus was first cultivated in 1910 by Twort and Ingram[2] from 4 cases on Dorset's egg medium to which dead tubercle bacilli or timothy hay bacilli (*Bact. phlei*) were added. The medium had the following composition:

| | |
|---|---|
| Egg, | 75 c.c. |
| NaCl (0.8 per cent), | 21 c.c. |
| Pure glycerin, | 4 c.c. |
| Dried *Bact. phlei*, | 1 oz. |

Later the organism was cultivated by Holth[3] from 2 cases in Denmark and by Meyer[4] from 8 cases in America. In Holth's work it was grown on various media, blood-serum containing 4 per cent. glycerin and 2 per cent. killed tubercle bacilli, serum containing $\frac{1}{4}$ volume liver broth, 2 per cent. dead tubercle bacilli and 4 per cent. glycerin, and serum agar containing 2 per cent. killed tubercle bacilli and 10 per cent. glycerin, as well as on Twort's medium. The best growth was in blood-serum with liver broth, dead tubercle bacilli and glycerin. Meyer cultivated it on media containing extracts of dead tubercle bacilli, on ordinary serum agar to which 2 per cent. dead tubercle bacilli and 4 per cent. glycerin were added, and on horse or ox serum containing 5 per cent. glycerin extract of dead tubercle bacilli. The organism was also obtained by McFadyean, Sheather, and Edwards[5] from 27 cases of paratuberculosis in South Africa.

The cultivated organisms have the following characteristics:

**Morphology.**—Like mammalian tubercle bacilli but somewhat thicker, about 1 $\mu$ long (Meyer) and tending to form club-shaped individuals.

**Staining Reactions.**—Acid-fast and alcohol-fast, staining like tubercle bacilli by Ziehl-Neelsen. Also stain well with hot anilin gentian-violet or carbolfuchsin, badly with carbol thionin or methylene-blue (Holth). Stain well by Gram if anilin gentian-violet be allowed to act one hour and by Giemsa (twenty-four hours' exposure) followed by tannic acid.

**Cultural Characters.**—Growth slow, only at 37° C. requiring four to six weeks before first appearance. No anaërobic growth.

*Serum Agar Containing 5 Per Cent. Glycerin Extract of the Tubercle Bacillus.*—Abundant mass of small, round, grayish-white colonies appearing after six to eight weeks. They remain the same size or in ten to twelve weeks grow to large, irregular, grayish-brown, dry, brittle colonies which show darker centers and large fluffy peripheries. Grow slower in stab cultures, grayish-white in the upper part.

*Agar Containing Glycerin Extract of Tubercle Bacillus.*—Irregular, clumpy, grayish-yellow mass of colonies or single colonies with yellow-brown nuclei and slightly wrinkled, crumbly, gray-yellow peripheral film, 2 to 4 mm. in size. Irregular growth in the upper part of stab cultures.

*Liver Broth Containing Blood-serum and Glycerin Extract of the Tubercle Bacillus.*—After four weeks a slight sediment at the bottom, gradually turning into a precipitate of irregular clumps of bacilli. Occasionally a grayish-white membrane forms along the sides of the glass container. Substratum clear and transparent. Superficial growth only from strains cultivated on proper agar and then transferred several times to broth, when an uneven, thick, superficial film slowly develops.

It has subsequently been shown by Twort and Ingram[6] that the substance necessary for the growth of Johne's bacillus is present in an alcoholic extract of the timothy hay bacillus (*Bact. phlei*) as well as in a glycerin extract. It is found in the human tubercle bacillus but not in the bovine bacillus. The wax and fatty acids of *Bacterium phlei* do not furnish the substance. Twort and Craig[7] report that Grassberger's butter bacillus, Dubard's fish tubercle bacillus, Binot's paratubercle bacillus and other acid-fast organisms (Tobler I and III) will also provoke the growth of Johne's bacillus.

**Pathogenic Properties.**—While Johne's disease has been produced experimentally in bovines by Twort and Ingram, Holth, Bang,[8] and Miesner and Trapp[9] the acid-fast organisms cultivated from it have very little pathogenic action on animals. Intravenous intraperitoneal and subcutaneous inoculation of rabbits, guinea-pigs, calves, hens, or mice does not produce anything like the natural disease (Twort and Craig).

Occasionally localized abscesses or caseous nodules are found on the serous membranes but the general health of the animal is not affected. Repeated intravenous inoculation is fatal to animals but there are no distinctive lesions at autopsy.

Twort and Ingram[10] report that the disease can be communicated to bovines by injecting pure cultures intravenously and intraperitoneally and by feeding, and to goats by intra-

peritoneal and intravenous inoculation. Apparently this was not confirmed by Twort and Craig.[11]

Lesions resembling Johne's disease have been reported in deer by McFadyean[12] and in sheep by Stockman.[13]

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2. Twort and Ingram: Proc. Roy. Soc., vol. 83, 84.
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### OLSCHANETZKY'S PARTIALLY ACID-FAST BACILLUS

Isolated by Olschanetzky from the pus of a liver abscess of a rat, *Mus decumanus*. In the pus stained by aqueous methylene-blue a slender rod was found which on cultivation on agar was partially acid- and alcohol-fast.

**Morphology** best made out in milk cultures. Rods arranged in bundles; V forms, clubbed forms, hooks, and branching organisms occur. The V forms and the branching are best demonstrated on agar with 3 per cent. NaCl but appear also on gelatin. Club-forms and hooks are best developed on ox serum and glycerin-ox-serum. Threads are also formed on ox serum, especially in old cultures and after repeated transfer on this medium. Non-motile.

**Staining Reactions.**—Stains with ordinary anilin dyes. Gram-positive. With Gram's stain the organisms are much thinner than when stained with carbolfuchsin. With methylene-blue they are still thinner. Granules are present in preparations stained by Neisser and Piorkowski. Stained by Ziehl-Neelsen the organisms show acid-fast areas and the granules demonstrated by Neisser's and Piorkowski's stains are deeply tinted. Resistance to decolorization fairly great. Alcohol has less action than 5 per cent. $H_2SO_4$. Greatest decolorizing action from 5 per cent. $H_2SO_4$ followed by alcohol, but by this method many granules remain stained.

In old cultures stained by Ziehl many entire rods remain stained, while in young cultures only the granular rods appear. On potato the acid-fastness is marked in young cultures but after ten days is lost and the rods tend to disintegrate.

**Cultural Characters.**—Grows under both aërobic and anaërobic conditions. Rapid and abundant development. Optimum temperature 37° C. Growth slow at 10° to 12° C. No growth in acid media.

*Agar Slant.*—Pearl-like round transparent expansion. After forty-eight hours the growth is more abundant, softer and turbid. Double-layered. Later the pearl-like masses coalesce, the growth looks milk-white and fatty and on the edges individual small pearl-like masses of growth appear. Comma-like extensions grow into the water of condensation which is not turbid but shows a dusty granular precipitate and individual granules swimming on the surface.

*Agar Stab.*—Abundant growth, gray-white in color along entire line of inoculation down to the bottom of the tube. After a couple of days the surface is covered with a fine white non-characteristic layer.

*Agar Plates.*—Small, raised, round colonies, dew-drop-white to gray:white in color. Under low power the surface colonies are transparent, slightly yellow, lobular, and finely granular. Deep colonies sharply limited, golden-yellow, darker in the middle than at the edges, oval to round, finely granular.

*Glycerin Agar.*—Growth like that on plain agar but more abundant.

*Agar* with 3 per cent. NaCl. Abundant growth.

Growth very slow in *gelatin*. No liquefaction.

*Gelatin Plates.*—Colonies visible after two days; round, circumscribed margins, light yellow in the middle, golden-yellow on the edges, finely granular.

*Glycerin Broth.*—Turbidity in twenty-four hours; white, fairly abundant precipitate which is easily dislodged and again sinks to the bottom. Later the precipitate seems to be made up of coherent films which cling fast to the bottom of the tube. After four days, a surface film which when transferred to fresh broth gives rise to surface films without turbidity. After three to four days turbidity and precipitate develop.

*Milk.*—Good growth, no coagulation. After five days, weak acid reaction. At the bottom an abundant gray-white precipitate.

*Glycerin Potato.*—In forty-eight hours a thin, slightly raised, whitish shimmering colony which clings to the potato and is dry and hard.

*Coagulated Ox-serum.*—Abundant double-layered soft growth limited to line of inoculation. Water of condensation not turbid.

Indol not produced. No gas from glucose, lactose, or saccharose.

Destroyed in two minutes at 70° C., instantaneously by boiling.

*Pathogenic* to rats by intraperitoneal inoculation which is followed by chronic abscess formation.

Olschanetzky: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1902, xxxii, Orig., 16.

### MYCOBACTERIUM SMEGMATIS (Alvarez and Tavel)

Described as *Bacillus smegmatis* by Alvarez and Tavel[1] from the smegma and found about the same time by Matterstock.[2]

**Morphology.**—Rod-shaped organisms, variable in size, lying on the desquamated epithelial cells of the smegma and in their neighborhood. Usually somewhat thicker than the tubercle bacillus. Non-motile.

**Stained** with some difficulty by ordinary dyes. Acid-fast, but not so strongly so as the tubercle bacillus. Gram-positive.

**Cultivation.**—Many attempts have been made to cultivate the smegma bacillus and several different strains of acid-fast bacilli have been obtained by different individuals.

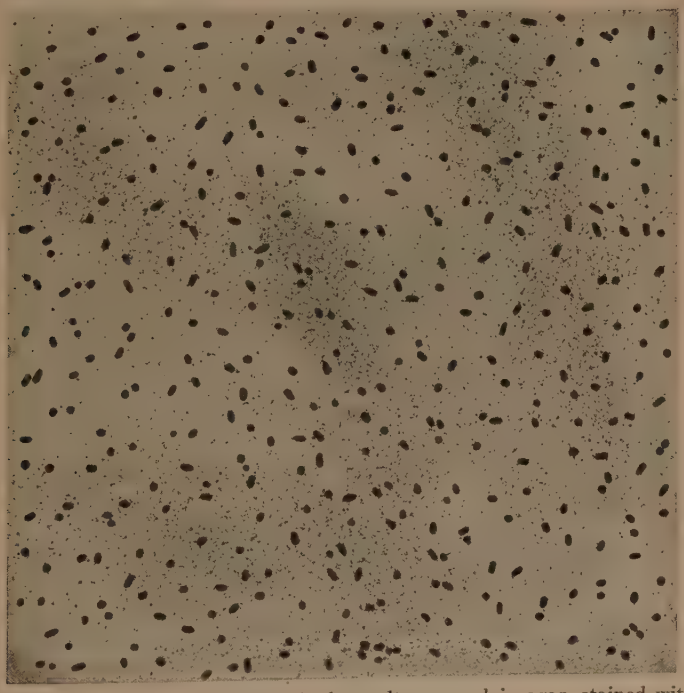

Fig. 8.—Acid-fast bacilli from smegma, six-day culture on plain agar, stained with carbol-fuchsin and methylene-blue.

These strains differ markedly in cultural reactions and in pathogenic action and there is some discussion as to which ones represent the true smegma bacillus present in the secretions.

Laser[3] cultivated organisms from the smegma on blood agar and glycerin agar. The colonies were small, like colonies of streptococci or diphtheria bacilli; like drops of dew. Little or no growth developed on plain agar, a better growth on glucose agar. Barely visible growth on potato. Microscopically these colonies showed rod-shaped organisms with terminal swellings which could be stained by methylene-blue, fuchsin, and Gram. The ends of

the organisms stained better than the central parts which were often colorless. Czaplewski[4] grew similar bacilli on Wassermann's nutrose agar. Growth best at 37° C. Impression-films from the growth revealed slender bacilli which looked like diphtheria bacilli and which retained Gram's stain. By double staining with carbolfuchsin, 5 per cent. $H_2SO_4$ and methylene-blue, the organisms were dark cherry-red. Fraenkel[5] states that if smegma be streaked on Heyden agar or nutrose agar and impression coverslips made at once and stained with hot carbolfuchsin, decolorized with hydrochloric acid and alcohol, and then stained with methylene-blue, they reveal larger and smaller numbers of slender bacilli, pointed at both ends, somewhat curved along the long axis, lying in and on the epithelial cells. They look like tubercle bacilli and show the characteristic light red arterial staining. These are the true smegma bacilli of Alvarez and Tavel and of Matterstock. With these organisms are diphtheria-like rods which are also red, but a deeper burgundy or like the color of venous blood, together with cocci stained blue. After twenty-four hours incubation fresh impression coverslips reveal cocci and diphtheroids. The latter are acid-fast along their entire length or contain acid-fast granules. Further cultivation gives only diphtheroid organisms with acid-fast granules. These diphtheroids are not the true smegma bacilli. The organisms cultivated by Laser and Czaplewski are these diphtheroids.

Moeller[6] cultivated acid-fast organisms from the smegma by blistering the skin with cantharides. The serum from the blister, incubated at 37° C., showed an increase of organisms. After three to four days a dry film developed on the surface. Bacilli were present in great numbers and cultures were obtained on glycerin agar plates. Similar organisms were cultivated by mixing the secretions with human serum. Moeller's strains had the following characteristics:

**Morphology.**—In young cultures the organisms showed great variation in size and shape. They were usually slender, slightly curved rods looking exactly like tubercle bacilli. In old cultures the organisms were plumper. Milk cultures showed pleomorphic organisms, rods with unstained vacuoles, club-shaped enlargements, threads, intensely-staining granules and "coccothrix" forms.

**Staining reactions** exactly like those of the tubercle bacillus. The organisms were absolutely acid- and alcohol-fast and were not decolorized by twelve minutes exposure to 3 per cent. HCl in alcohol. They could be stained in the cold with dilute carbolfuchsin. When stained by the methods of Bunge and of Pappenheim they reacted like the tubercle bacillus. The alcohol and acid-fastness were not lost on cultivation, organisms in the twenty-fifth generation reacting like the original.

**Cultural Characters.**—Extremely aërobic, growing abundantly under aërobic conditions, sparsely under anaërobic conditions. In first generations growth was slow at 37° C., colonies appearing visible to the naked eye only after three days. After repeated transfer the organism became accustomed to artificial media and grew much more rapidly, colonies appearing in twenty-four hours. Growth at room temperature slow. Growth on all the usual media.

*Glycerin* agar colonies at 37° C. small, lustreless, grayish-white dry disks with rounded edges. Later they become folded and form a velvet-like mass with greater lustre. At room temperature the growth is apt to remain dry. Water of condensation clear with a film on the surface which runs up the glass wall.

*Potato.*—Abundant development as a gray-white dull glistening mass.

*Milk.*—Rapid abundant growth without coagulation or change in color.

*Broth.*—Dry white film on the surface, running up the glass, the broth remaining clear. On shaking small fragments break off sinking to the bottom.

**Pathogenic Action.**—Moeller found that pure cultures of his strains were not virulent to rabbits, guinea-pigs, hens, and doves. This was confirmed by Aujeszky[7] who found that the intraperitoneal injection of cultures together with sterile butter or olive oil was occasionally pathogenic to rabbits. Out of 5 rabbits, one had an abscess, another died in twenty-six days with the abdominal cavity full of purulocaseous nodules the size of lentils, containing acid-fast bacilli in large quantities which could be brought to cultivation. When the organisms were introduced into the body of frogs they lost their acid-fastness.

1. Alvarez and Tavel: Arch. de physiol. norm. et path., 1885, 3. s., vi, 302.
2. Matterstock: Sitzungsb. d. phys.-med. Gesellsch. zu Würzb., 1885, v.
3. Laser: München. med. Wchnschr., 1897, xliv, 1191.
4. Czaplewski: München. med. Wchnschr., 1897, xliv, 1192.
5. Fraenkel: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1901, xxix, Orig., 1.
6. Moeller: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1902, xxxi, Orig., 278.
7. Aujeszky: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1907, xxvi, 415.

### MYCOBACTERIUM BEROLINENSE Petri-Rabinovitsch

Isolated by Rabinovitsch[1] from market butter in Berlin and Philadelphia by the intraperitoneal injection of guinea-pigs. Usually known as the "butter bacillus."

**Morphology.**—Non-motile rods sometimes slightly curved, usually single. When rapidly growing in the tissues they frequently form rows of parallel organisms. Occasionally they grow out as long unbranched threads, at times tending to break up irregularly into short segments. They are thicker than tubercle bacilli and may show lateral, somewhat club-shaped enlargements.

No spores are produced, but intensely staining granules may be present inside the cells.

**Staining Reactions.**—In fresh preparations the organisms take the stain by Ziehl-Neelsen's and Ehrlich's methods, *i. e.*, are acid-fast. Often they can not be differentiated from tubercle bacilli. Organisms from old cultures after a year on artificial media are also acid-fast. Soaked in absolute alcohol forty-eight hours without fixation, stained with carbol-fuchsin and then treated one to two minutes with dilute $H_2SO_4$, washed with water and stained with methylene-blue, they remain acid-fast just like the tubercle bacillus. They also resist decolorization four minutes in 6 per cent. $H_2SO_4$ after fixation in 5 per cent. chromic

Fig. 9.—Butter bacillus from culture, stained with carbolfuchsin.

acid and staining with carbolfuchsin. Stained with very dilute aqueous methylene-blue solution without warming they appear as homogeneously stained rods containing at times deeply stained granules, while the tubercle bacillus stained in the same way shows very darkly stained granules, the body of the organism remaining unstained. In sections from infected tissues after long hardening in alcohol the butter bacilli are not so acid-fast as true tubercle bacilli. Cultivated on butter agar to determine the influence of fat, they are acid-fast, while central preparations of *Bacillus pyocyaneus*, *Bacillus prodigiosus*, *Staphylococcus aureus*, and *Bacillus coli* cultivated on the same medium are not acid-fast.

If the fat be removed from the organisms by treatment with potassium hydrate and ether, or alcohol and ether, they are still acid-fast. Occasionally, however, in smears from animals dead of peritonitis, one can find non-acid-fast rods after the fat has been removed, and also similar organisms in cultures.

**Cultivation.**—The butter bacilli may be cultivated without difficulty, better from animals which die after their lesions are well developed than from animals killed after a short time.

On *agar* the growth at first is a thick, moist, cream-like mass, later the surface of the agar is covered with a deeply folded membrane which frequently has an orange or copper color. After frequent passage through the animal body the organisms form a dry mass full of cracks, which often turns into a deeply wrinkled membrane closely resembling the growth of the tubercle bacillus on glycerin agar.

On *agar plates* the deep colonies are gray, round to oval, uniformly granular. Surface colonies develop better and are composed of uniformly granular gray centers and light peripheral zones which are made up of twisted or wave-like curved branches. The surface of the colonies is often dry and dome-like.

On *butter-agar* the young colonies are small, white, and dry, later the whole surface is covered by an orange- to copper-yellow mass. Microscopically the organisms here are extremely small.

On *potato* the growth is abundant, moist, and gray.

In *gelatin stabs* the growth is slow at room temperature, small isolated colonies appearing along the line of inoculation on the third day. No liquefaction.

Growth rapid in *broth* and *glycerin broth*, an abundant wrinkled or folded surface membrane forming after two to three days, the broth remaining clear. This growth resembles that of the tubercle bacillus.

*Milk* not clotted; on the surface an abundant yellowish-red layer, lying in a ring along the glass wall.

The odor of the butter bacillus cultures is unpleasant and ammoniacal while that of the true tubercle bacillus is pleasant and like the scent of flowers. Indol produced in small quantities (absent in tubercle bacillus cultures).

In Prorkmann's colorless protein-free media the butter bacilli grew readily, growth appearing on the second or third day. In about ten days the fluid medium is covered with a thick, yellow, dense, folded, wrinkled membrane while the medium takes on the yellow color of ordinary broth. On this medium the tubercle bacillus produces a thin membrane covering the surface only after about ten days, the folding of the growth developing later. If acid be added the tube containing the tubercle bacillus remains clear while that containing the butter bacillus shows an outspoken turbidity. The latter organisms, furthermore, produce large quantities of alkali; at times, however, the reaction may change and acid production set in.

The butter bacillus like the tubercle bacillus contains fat, turning deep black on treatment over night with Flemming's solution. Treated with cold alcohol or cold ether the fat is not extracted, such organisms also staining black with Flemming's solution. The fat can be extracted with sodium hydrate and alcohol in twenty-four hours, after which the organisms take on a yellowish-green color with Flemming's solution.

In general the growth of the butter bacillus is more abundant than that of the tubercle bacillus, takes place at room temperature, has a yellow or copper color, an unpleasant odor, forms traces of indol and produces chemical changes in the media which are not found in cultures of Koch's bacillus.

**Pathogenic Action.**—In guinea-pigs inoculated intraperitoneally with butter containing these acid-fast organisms, the lesions could easily be mistaken for true tuberculosis. Animals killed three to four weeks after intraperitoneal injection have a peritonitis varying from a slight fibrinous exudate to a layer of connective tissue on the abdominal organs. The entire peritoneum and mesentery are permeated with nodules which are also present in the wall of the gut. The mesenteric glands are swollen, sometimes with purulent, caseous contents. The surface of the liver is studded with nodules which sometimes look like foreign bodies in normal liver tissue and only occasionally are found in the liver parenchyma. The spleen is enlarged and filled with nodules which are also present on the surface of the kidneys. The lungs have the same nodules on the surface but not in the interior. Sternal glands swollen, not caseous.

The butter bacilli are present everywhere in the glands and in the nodules in the various organs and can be cultivated without difficulty. They are present but sparsely in the blood but may be obtained on culture. Animals which are injected with small quantities of butter containing the organisms may recover, after a time gaining in weight and assuming a normal appearance.

In pure culture Rabinovitsch's bacillus is also pathogenic to guinea-pigs but not in all cases. It is not pathogenic to rabbits or white mice. Of 40 injected intraperitoneally, 6 died after four to eight weeks. At autopsy they show peritonitis varying from slight flecks of fibrin to an abundant growth of connective tissue. The mesentery shows small nodules, the glands are much enlarged but not caseous. The liver is covered with miliary nodules. The chest organs usually show no lesions, but occasionally the lungs may show minute miliary nodules. In general the lesions produced by pure cultures are not so marked as with the butter containing the organisms, and their number in the lesions is not so great. They can always be cultivated however.

Microscopically the nodules are made up of lymphoid elements, epithelioid cells and polynuclear cells in small number. The acid-fast organisms are present in the centers of the fresh nodules, also on the edges of the caseous areas. Frequently the nodules become purulent with necrotic material in the center and a fringe of pus cells on the periphery. The process is more exudative than proliferative in character. The typical giant-cells of true tuberculosis were not found by Rabinovitsch but occasionally so-called polynuclear giant-cells were observed, especially in the animals injected with butter containing the organisms. These had nuclei lying centrally and were like the cells seen in young granulation tissue.

The lesions in animals were regarded by Rabinovitsch as pseudotuberculosis, resembling glanders more than true tuberculosis.

The Rabinovitsch organism was also isolated by Petri[2] in market butter from Berlin. The cultural characters were practically the same. In sections from infected tissues hardened in alcohol the organisms stained blue by Ziehl's method and only a few organisms showed a slight tendency to take the red stain. They were arranged in clumps and were never found in giant-cells.

It was subsequently shown by Meyer[3] that if the organisms were introduced with butter into the peritoneal cavity of guinea-pigs and rabbits nodules developed which after four days contained threads of fibrin, drops of fat and acid-fast bacilli arranged in rings or radially. Many of the organisms showed Y-shaped branching. After five weeks the nodules had a characteristic appearance. The centers were made up of radially arranged bacteria in amorphous masses of reddish material, surrounded by a ring of intensely stained polymorphonuclear leukocytes, then another amorphous mass and finally a broad zone of small round mononuclear cells and connective tissue threads. Aujeszky[4] found that gray mice could be infected with large quantities of organisms and that intraperitoneal inoculation in frogs produced a kind of pseudotuberculosis. Lubarsch[5] reports that if the organisms be injected directly into the kidneys of rabbits they show a characteristic radial arrangement and clubbed extremities.

1. Rabinovitsch: Ztschr. f. Hyg., 1897, xxvi, 90.
2. Petri: Arb. a. d. K. Gsndhtsamte, 1898, xiv, 1.
3. Meyer: Centralbl. f. Bakteriol. [etc.], 1899, xxvi, 321.
4. Aujeszky: Centralbl. f. Bakteriol. [etc.], 1907, xxxvi, 415.
5. Lubarsch: Ztschr. f. Hyg., 1899, xxxi, 187.

### MYCOBACTERIUM PHLEI (Moeller)

Obtained from timothy hay by Moeller[1] and described as the "timothy-hay bacillus or grass bacillus No. 1."

**Morphology.**—Rods like the tubercle bacillus, with occasionally club-shaped enlargements at one or both ends. They are often curved and show more deeply stained granules wider than the organism. On many media they appear short and plump, as on serum media. In milk they are slender and not to be distinguished from tubercle bacilli, in gelatin fairly long. Gram-positive. Non-motile. True branching rare (Lubarsch[2]).

**Staining Reactions.**—Acid-fast and alcohol-fast from all media. Well stained by Ziehl-Neelsen. Cultivated on certain media and stained well with hot carbolfuchsin they are not decolorized by several minutes exposure to 40 per cent. $H_2SO_4$ and also resist a subsequent treatment with alcohol. Not decolorized by 3 per cent. HCl in alcohol. Both young and old organisms possess great acid-fastness. The organisms are also acid-fast in the sections from tissues.

*Cultivated* on ordinary media, best under aërobic conditions and at 37° C.

**Cultural Characters.**—*Glycerin Agar Plates.*—Gray-white, non-adherent, dry, scale-like colonies. They can be raised "in toto," are brittle, and difficult to rub up.

*Glycerin Agar Slant.*—Colonies develop along the line of inoculation, touching each other and having a scale-like appearance. They are at first white, then gray-white and after three to four days yellowish. Water of condensation remains clear, the growth extends over it and up the glass wall. This dry growth, slightly raised from the surface, is later swollen up and spreads irregularly at the periphery. In its growth it resembles the tubercle bacillus.

*Broth* cultures remain clear. Small crumbly masses develop along the glass wall and on the bottom, which on shaking are easily dislodged. A thin, delicate, transparent, yellowish-white film develops in three to four days on the surface. This is dry and lustreless and mounts up along the glass wall for 2 to 3 cm. On shaking, small fragments may be dislodged and sink to the bottom.

In *glycerin-saccharose-broth* the growth is the same as in plain broth. The membrane has more of a red or orange color and also mounts up along the walls of the tube.

On *potato* at 37° C. a thick yellowish mass forms which is warty, studded with numerous small nodules, all of which are dry and difficult to rub up. Potato offers the best medium for the growth of the organism.

*Litmus Milk.*—Growth is evident in about five days when small reddish-yellow masses appear at the edge of the cream layer. These are made up of bacilli. Later a larger yellowish ring is formed. After eight days the reaction is acid.

*Gelatin Stab.*—Growth in the stab in two to three days. After five days a dry surface expansion 0.5 mm. in diameter. After thirteen days growth along the stab to the bottom, with a dry uneven reddish-yellow surface expansion 0.3 mm. in diameter. No liquefaction. No change in color.

Growth rapid in the presence of oxygen. Under anaërobic conditions at 37° C. very small colonies develop after eight days.

*Ascites Agar Stab.*—At 18° C. development begins in twenty-four hours; after forty-eight hours moderate growth appears in the upper third of the stab with a surface expansion 0.5 mm. in diameter. After three days the surface growth is 0.75 mm. wide. After five days there is good growth along the stab to the bottom and the surface expansion is irregular, dry, lustreless, very uneven, prominent, light red in color and has a diameter of 2 mm. After thirteen days the surface is puffed up and has a diameter of 3 mm. like tubercle bacillus cultures. The chamois color is like the color of mammalian and avian tubercle bacillus cultures on solid media. No change in color of the medium.

*Coagulated Beef Serum Slants.*—In twenty-four hours many small colonies along the line of inoculation; in forty-eight hours larger, many 0.5 mm. in diameter, always isolated. On the third day the colonies form a very thin but quite evident streak. After five days the colonies are still larger, the largest 0.5 to 0.75 mm. in diameter. No liquefaction. No change in color of the medium.

*Sugar Beets.*—A thin dry yellowish-red mass develops along the line of inoculation. After two to three days this mass consists of crowded or isolated upright colonies which are often higher than the diameter at the base. After fourteen days the growth is abundant and confluent, individual colonies rising from it at right angles.

In *serum agar stabs* at room temperature or at 18° C. growth appears in twenty-four hours. After forty-eight hours the upper third of the stab is permeated with growth while at the point of puncture an expansion appears, 0.5 mm. in diameter. After three days this is 0.75 mm. in diameter. After five days growth appears along the entire stab and the irregular, dry, lustreless, prominent reddish-yellow surface expansion is 1.5 to 2 mm. in diameter. After thirteen days this is about 2 to 3 mm. wide. The medium is not changed in color.

**Pathogenic Action.**—The lesions resemble those seen in true tuberculosis. Pathogenic to guinea-pigs by intraperitoneal inoculation of large doses, 3 to 4 c.c. of broth or milk cultures. The animals may die in two to three days, the organisms being always present in the blood in various parts of the body. In animals dying after five to six weeks there is infiltration at the site of inoculation with a caseous mass in the center, fluid exudate in the pleura, enlarged lymphatic glands, and many small grayish-white to yellow nodules in mesentery and peritoneum. The spleen is swollen with a moderate number of grayish-white nodules on the surface. Similar nodules occur on the surface of the liver and also in the lungs. The nodules may be yellowish, and are from pin-head to lentils in size. Small cavities filled with caseous material may form in the lungs. From all the infected tissues the organism can be demonstrated by staining reactions and obtained in pure cultures. Microscopically the nodules show acid-fast organisms, and resemble tubercles. The lungs show a kind of catarrhal pneumonia with caseation. The organism is also virulent to rabbits, the lesions being the same as in guinea-pigs. The nodules, however, show true giant-cells microscopically.

Lubarsch[2] subsequently showed that if the organisms be injected directly into the kidney of rabbits and part of the kidney excised after thirteen days, the tissue near the injection contains yellowish-white relatively hard masses the size of lentils, and the capsule of the kidney is studded with caseous material. Microscopically the lesions look like true tubercles with clubbed organisms radially arranged, lying in Langhans' giant-cells or surrounded by a wreath of polymorphonuclear leukocytes. If the rest of the kidney be taken out at the end of thirty-one days the tubercles show more Langhans' giant-cells and fewer epithelial cells, the centers being made up of caseous material.

When the organisms are injected intraperitoneally with butter the lesions in guinea-pigs show radial arrangement of branching threads with club-shaped enlargements (Meyer[3]). The lesions are made up of epitheliod cells with one or more nuclei, Langhans' giant-cells and small caseous masses. Many acid-fast bacilli are present. In rabbits killed after eight weeks fairly large crusts are found containing round or oval caseous masses which often coalesce, round cells, and badly stained bacilli in capsules of connective tissue. Typical "actinomyces Drusen" have also been observed by Lubarsch following intra-arterial injection.

1. Moeller: Deutsche med. Wchnschr., 1898, xxiv, 376.
2. Lubarsch: Ztschr. f. Hyg., 1899, xxxi, 187.
3. Meyer: Centralbl. f. Bakteriol. [etc.], 1899, xxvi, 321.

### MYCOBACTERIUM STERCUSIS (Moeller)

Cultivated by Moeller[1] from the dejecta of cows, horses, goats, swine, and mules, and described as the "mist bacillus."

**Morphology.**—Slender bacilli 1 to 4 $\mu$ long and 0.2 to 0.4 $\mu$ wide. Often slightly curved, frequently in threads of two or three elements. Inside the organisms are often deeply stained granules whose diameter is greater than that of the bacillus. The organism sometimes shows club-shaped enlargements on one or both ends.

On serum media the organisms are short and straight. They are frequently divided into short fragments (coccothrix form). On gelatin media the organisms are usually thicker. In glycerin broth they are short and straight, also frequently curved. They are arranged in Y forms at obtuse angles, and behind each other in twos and threes. Frequently two organisms are found side by side with one pushed ahead of the other. In mixed cultures the organisms are often longer and larger and show lateral clubs on one or both sides more frequently than usual and long threads which are always unbranched.

**Staining Properties.**—Alcohol- and acid-fast, especially in young cultures. Old organisms stained by Ziehl-Neelsen or Fraenkel-Gabbet are frequently partially decolorized, particularly old organisms on potato media. The organisms are especially acid-fast in young cultures on serum agar, ascites, agar, and glycerin broth. In the last medium individual bacilli are often decolorized and take methylene-blue. Preparations may often be obtained, however, which after deep staining with carbolfuchsin cannot be decolorized with 40 per cent. $H_2SO_4$ in one minute, followed by strong alcohol solutions. With cold carbolfuchsin staining is uncertain. Excellent preparations obtained by Czaplewski's tubercle bacillus method.

Fig. 10.—*Mycobacterium stercusis* (mist bacillus of Moeller), film from culture stained with carbolfuchsin and Gabbet's methylene-blue.

*Cultivated* easily on ordinary media. Optimum temperature 37° C.

**Cultural Reactions.**—*Glycerin Agar Plates* at 37° C. show in two to three days small, raised colonies, at first grayish-white, later turning yellow in the middle. Frequently the centers show button-like projections which often sink in in crater form. The colonies are round with almost sharp edges. Originally dull, they are later moist and glistening, the larger colonies showing concentric ring-formed structures with sharply limited edges. The colonies are beautiful ochre-yellow.

*Sugar Beets.*—In two days a thin white growth extends along the line of inoculation, gradually turning sulphur-yellow. After three to four days the growth shows areas of golden-yellow.

*Potato.*—Colonies develop on raw and cooked potato at 37° C. in one to two days. They are at first whitish-gray, later grayish-yellow to ochre-yellow, raised high from the surface, slightly glistening.

*Agar Slant.*—Isolated raised colonies in forty-eight hours at 37° C., later coalescing. With heavy inoculations a broad, somewhat glistening mass with sharp edges develops along the streak. This mass is first grayish-white, then yellow. The growth is not limited to the line of inoculation, but gradually broadens, always, however, with sharp edges. In old cultures

a wave-like extension appears at right angles to the long axis of the streak which often leads to the formation of double layers. Bits of film float on the water of condensation and a viscid sediment develops, otherwise the water remains clear.

On *glycerin agar streaks* the growth is orange to golden-yellow, otherwise the same as on plain agar.

*Agar Stab.*—Good homogeneous growth along the stab, grayish-white in color. On the surface a grayish-yellow glistening mass which spreads out and frequently shows radial extensions. After three to four days the whole surface is covered with a yellow easily lifted mass.

*Coagulated Serum Slants.*—At 37° C. in two days and at 20° C. in five days a delicate slightly yellow growth along the streak. Microscopically the baçilli appear in bundles on this medium in impression coverslips.

*Serum Agar Stab.*—In two to three days abundant growth along the line of inoculation extending to the bottom.

*Ascites Agar Stab.*—In two or three days a very abundant growth along the line of inoculation to the bottom.

*Milk.*—Growth visible in four to five days with occasionally a coagulation. The milk assumes a yellowish-red color with an ochre-yellow ring growth on the surface, increased in eight days.

*Broth.*—In two days at 37° C. an abundant development with a yellow turbidity. A rich, golden sediment soon forms which on shaking rises up in skeins; later a surface membrane appears which then sinks in the form of small floccules. These floccules may adhere to the glass wall like coagulated albumin and are dislodged on shaking; microscopically the skeins and floccules give beautiful pictures of bacilli arranged in parallel rows.

In glycerin broth the growth is more abundant and rapid than on ordinary broth. In glycerin saccharose broth turbidity and sediment develop.

*Gelatin.*—Grayish-white growth along the stab in five to six days. Very slight growth on the surface. No liquefaction. Abundant growth in glycerin gelatin.

Abundant growth in dung extract filtered through Chamberland filters.

No indol. No specific odor in broth.

**Pathogenic properties** like those of the timothy hay bacillus (*Mycobacterium phlei*). The organism is virulent to guinea-pigs and rabbits, especially by intraperitoneal injection. In guinea-pigs the serous membranes and the organs are studded with grayish-white to yellowish nodules which microscopically show the acid-fast organisms and occasional giant-cells. Giant-cells with nuclei arranged about the wall were observed only in rabbits. The pathogenic changes resemble closely those of true tuberculosis. Lubarsch[2] regards the lesions in rabbits as not exactly like those from the timothy hay bacillus. The masses are star-shaped or radially arranged and show more large single cells than epithelioid cells. When the organisms are injected directly into the kidney the lesions show radially arranged bacilli with clubbed ends, sometimes in giant-cells, sometimes surrounded by leukocytes. Not virulent to mice, frogs, and pigeons (Aujeszky[3]).

1. Moeller: Deutsche med. Wchnschr., 1898, xxiv, 376.
2. Lubarsch: Ztschr. f. Hyg., 1899, xxxi, 187.
3. Aujeszky: Centralbl. f. Bakteriol. [etc.], 1907, xxxvi, 415.

### MYCOBACTERIUM GRAMINIS (Moeller)

Described by Moeller[1] from plant dust on cultivated soil as "grass bacillus No. 2."

**Morphology.**—The majority of the organisms are at first coccus-like bodies and rods about 1 to 5 $\mu$ long and 0.2 to 0.4 $\mu$ wide. Longer forms are present in the nodules of infected guinea-pigs. They are usually slightly curved. They are arranged as Y forms, at obtuse angles and in twos and threes. On solid media, especially at the edges of glycerin agar cultures kept three to four days at 37° C., long threads appear, sometimes branched. Some of these threads show a peculiar fragmentation (coccothrix) which is especially evident in milk cultures. The threads show deeply stained granules wider than their diameter. The bacillary forms may be swollen at one or both ends. The branching forms are usually long threads from which at right angles on both sides long thread-formed branches are given off, as well as shorter branches with club-shaped extremities. The branches may often give off further branches. Motile when young. True branching reported by Lubarsch.[2]

**Staining Reactions.**—In young cultures the organisms stain like the tubercle bacillus, being both acid- and alcohol-fast. They may be stained by various tubercle bacillus stains, Fraenkel's, Ehrlich's, Czaplewski's, Babbet's and Ziehl-Neelsen's. In old cultures the organisms are not so alcohol-fast as in young cultures and show a pale rose color. Gram-positive.

**Cultural Characters.**—*Glycerin Agar Slants.*—Growth is abundant at 37° C. In two days small, delicate, dew-drop-like colonies appear which later coalesce. The growth is

raised, dull and glistening and often has a yellow color. The water of condensation remains clear, but on the surface small films develop which sink to the bottom.

*Potato.*—Abundant development at 37° C. in the form of a thick grayish-white mass.

*Milk.*—Rapid growth, the milk turning acid in two to three days.

*Broth.*—After three to four days at room temperature a viscid sediment forms, with a whitish-gray surface film running up the glass. The broth remains clear and has no odor.

*Gelatin Stabs.*—After four to five days at 20° C. a thick grayish-white growth appears along the line of inoculation and on the surface. No liquefaction.

**Pathogenic Action.**—Virulent to guinea-pigs, which die four to six weeks after intraperitoneal inoculation. The lesions resemble those produced by the true tubercle bacillus and the caseous masses contain large numbers of the characteristic acid-fast organisms. Microscopically the lesions resemble true tuberculosis. According to Lubarsch[2] if the organisms be injected directly into the kidney of rabbits, they produce interstitial nephritis with

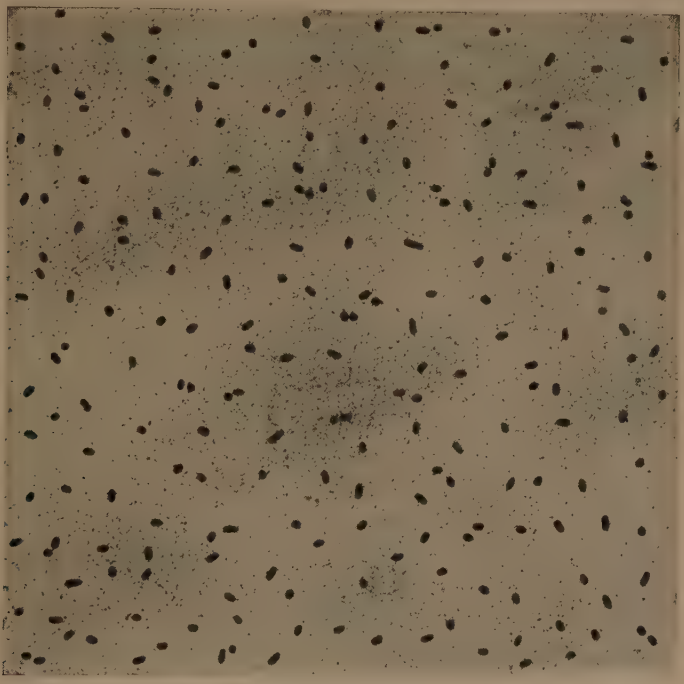

Fig. 11.—*Mycobacterium graminis* (Grass bacillus of Moeller), six-day culture, stained with carbolfuchsin.

many large epithelioid cells and few polymorphonuclear leukocytes. No radial arrangement. Other animals, however, may show radial arrangements and clubbed extremities on the organisms.

Aujeszky[3] has produced pseudotuberculosis by intraperitoneal injection of this organism, but local abscesses only by subcutaneous inoculation. Intravenous injection had no effect. Frogs injected with large quantities intraperitoneally died at the end of fourteen to sixteen days with typical pseudotuberculosis. The organisms could be isolated from the blood and from the pseudotubercles, but experience a loss of acid-fastness from residence in the frog.

1. Moeller: Centralbl. f. Bakteriol, [etc.], 1899, xxv, 369.
2. Lubarsch: Ztschr. f. Hyg., 1899, xxxi, 187.
3. Aujeszky: Centralbl. f. Bakteriol. [etc.], 1907, xxxvi, 415.

### MYCOBACTERIUM RUBRO-PERTINCTUM (Hefferan)

First isolated by Grassberger[1] from butter and named *Bacillus rubro-pertinctus* by Hefferan.[2]

Rods 1.5 to 3 $\mu$ in length, on 24 agar cultures, longer in old cultures. According to Grassberger not acid-resistant.

Found by Hefferan to resist decolorization with 10 per cent. $H_2SO_4$ after staining with hot carbolfuchsin. Shorter and thicker than the tubercle bacillus.

*Agar colonies* grow slowly and are just visible to the naked eye in five days, irregular, granular.

*Agar Slant.*—Lustreless growth, dry and spreading, later wrinkled and vermilion-red.

*Gelatin colonies* on the surface irregular, folded, crenated and red, in the depths ovoid and granular.

*Gelatin Stab.*—Faint growth along line of inoculation; surface growth as an orange-red colony. No liquefaction.

*Potato.*—Orange-red, rough, granular, moist growth in ten days.

*Broth.*—Salmon-pink pellicle.

*Litmus Milk.*—Pink pellicle.

No gas production. Growth at 37° C. Aërobic. Indol found by Grassberger, not by Hefferan.

1. Grassberger: München. med. Wchnschr., 1899, xlvi, 382.
2. Hefferan: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1903, xi, 311, etc.

### MYCOBACTERIUM FRIBURGENSE (Korn)

Isolated by Korn[1] from market butter in Freiburg by the injection of small quantities intraperitoneally in guinea-pigs and called "butter bacillus No. 1." The organs showed small bacilli like tubercle bacilli when stained by Ziehl-Neelsen's method. Cultivated without difficulty on ordinary media.

**Morphology.**—In broth the organisms are like *Bacterium coli*, but often longer and somewhat curved. On agar the rods are thinner. In old agar and serum cultures the bacilli are segmented (coccothrix forms). The organisms are smaller when cultivated at room temperature than at 37° C. On potato great variations in morphology are seen. One can find coci, diplococci, many short rods, slightly-curved bacilli of variable thickness, often structures which resemble broken up actinomyces "Drusen," or the heads of spermatozoa, and drumstick forms. In sweet cream at 37° C. one finds chiefly bacilli, cocci, diplococci, and many short rods, but not the actinomyces-like forms. On cooked turnips at 37° C. normal rods appear in the first twenty-four hours while on the third or fourth day the rods break up and the preparation looks like a staphylococcus film. In infected animals the organism appears as bacilli not to be differentiated from tubercle bacilli, as longer threads and as rods resembling *Bacterium coli*. Branching observed in one instance by Korn in a culture on cooked rice at 37° C. from a purulent gland from a mouse. Non-motile. No spores.

**Staining Reactions.**—Stains badly with ordinary anilin dyes (1–100). Stains well with anilin gentian-violet, anilin or carbolfuchsin especially when warmed. Gram-positive when the organisms take the anilin gentian-violet after warming. Stains with Ziehl-Neelsen from all media, but the organisms are not so resistant as the tubercle bacillus. Resists decolorization by 10 per cent. HCl in alcohol one minute. In young cultures stains uniformly with dilute aqueous methylene-blue. In old cultures it shows irregular staining (coccothrix forms) like human and avian tuberculosis. The organisms retain acid-fastness in sections and in pure cultures preserved in 5 per cent. formalin and in absolute alcohol several weeks.

**Cultural Characters.**—*Gelatin stabs* at 37° C. After three days a white, slightly raised, slightly irregular dull mass which increases in size and is little characteristic. In old cultures the gelatin on the surface is often milky and turbid. Along line of inoculation a scanty development. No liquefaction.

Deep *gelatin* or *agar colonies* are round or oval, uniformly granular. Surface colonies at 37° C. roundish, grayish white, peripheral zone later raised, centers depressed.

*Glycerin Agar Slant.*—In twenty-four hours at 37° C. moderately thick, white, glistening mass with growth also in the water of condensation. After two to three days this develops thick swollen folds and a film forms on the surface. This film is at first thin and delicate, later thicker, running up the glass. After a time the entire surface of the agar is covered with a hard, folded membrane. As the agar dries the growth takes on a light copper color, most rapidly when the whitish cultures at 37° C. are transferred to room temperature.

*Broth* (with peptone).—A rather thick film forms on the surface in two days at 37° C. On shaking, parts of this film fall to the bottom and a new film forms. Growth in neutral or alkaline broth, but not in acid broth. Rapid growth in glycerin broth.

*Fermentation Tubes.*—No growth in closed arm. Growth in bulb like that in broth tubes. No gas from glucose.

*Milk.*—Good growth without change in reaction for six days. After this time the milk turns gray and thin and a moderate sediment forms. No clotting or peptonization. Whole milk or sweet cream shows a bright copper color along the edges. Growth on glycerin-horse-

blood-serum slower than on glycerin agar, after eight to ten days slowly turning bright orange. On asses blood-serum without glycerin, growth is slower than on glycerin agar and assumes a yellowish color.

On these two media the swollen folds seen on agar do not develop and the growth does not increase much in breadth.

*Potato.*—At 37° C. in twenty-four hours no growth visible. After forty-eight hours a dull slightly raised whitish mass appears which is slightly indentated, and has a brown color. The pigment seen on agar does not develop.

Growth also very good on boiled turnips of several varieties and on cooked rice. Pigment formation on cooked rice and red turnips, not on the other varieties of turnips.

Optimum temperature 37° C. Slow growth at room temperature.

Growth sparse at 37° C. under anaërobic conditions in an atmosphere of hydrogen and in deep cultures, none at room temperature.

Strong disagreeable odor from cultures which is difficult to describe and is not ammoniacal. Indol formation weak in glycerin broth and not always observed in broth without glycerin. Alkali formed in broth with and without glycerin.

Viability good. Cultures two months old kept in diffuse light at room temperature and then transferred to glycerin agar are still alive. Not destroyed by twelve-hour exposure to direct sunlight.

**Pathogenic Action.**—Not virulent by feeding to rabbits, hens, guinea-pigs, pigeons, white rats, or mice. Not virulent to rabbits and guinea-pigs by intraperitoneal or subcutaneous injection, as far as general infection is concerned. Occasionally abscesses were produced at the site of inoculation as large as a walnut and containing acid-fast organisms in pure culture. After six weeks the organisms were still acid-fast, but when transferred to glycerin agar produced only a few colonies, the majority being dead. The internal organs were always normal. Injection into the breast muscle of pigeons gave rise to a moderate circumscribed necrosis containing many bacilli. With hens a similar necrotic focus developed along the line of injection the size of a mark-piece, with a color like that of egg-yolk. These necrotic masses contained well stained bacilli which after six weeks were no longer visible. Intraperitoneal injection of white rats in larger quantities was followed by the formation of small nodules in the omentum. These became caseous and contained the organism in great numbers. All the other organs were normal. With subcutaneous injection of large quantities of suspensions of agar cultures, nodules developed at the site of inoculation the size of millet seeds. No reaction from small quantities injected subcutaneously or intraperitoneally. Intraperitoneal injection of white mice was always followed by the death of the animal in four to six weeks. In the animals dead in the shorter time the visceral and parietal peritoneum was studded with gray transparent submiliary nodules. Similar nodules were also present along the vessel walls in the subcutaneous tissues, on the walls of the bladder, in the spleen, in the kidneys and in the serous coats of the heart. No nodules in the liver. No changes in the lungs.

Microscopic preparations of all the organs (except the liver and lungs) show numerous acid-fast organisms which grow out on glycerin agar.

In mice dying after longer intervals yellowish-white or white nodules, miliary to millet seed in size were present everywhere, in the liver, kidneys, spleen, mesentery, bronchial glands, and underneath the skin. These nodules showed acid-fast organisms microscopically which were easily brought to cultivation on glycerin agar. No changes in heart and lungs. Subcutaneous injection was followed by the formation of pea-sized abscesses at the site of inoculation, containing thick fluid pus and acid-fast organisms, but no other bacilli. The animals usually died in three to six weeks without lesions in the internal organs. Rarely the abscesses healed. Occasionally subcutaneous injection was followed by the same type of lesions as are seen with intraperitoneal inoculation. Microscopically the nodules showed great similarity to caseation. The connective tissue was not so abundant and no giant-cells were present.

Korn's observations on animals were confirmed by Aujeszky[2] on rabbits, guinea-pigs, and white mice. One frog died from an intraperitoneal inoculation, showing a fibrinous exudate and enlarged lymph-glands. The organisms cultivated showed a loss in acid-fastness.

The organism is regarded by Korn as distinct from those described by Petri and Rabinovitsch and may be a kind of "pseudotubercle" bacillus.

1. Korn: Centralbl. f. Bakteriol. [etc.], 1899, xxv, Orig., 532.
2. Aujeszky: Centralbl. f. Bakteriol. [etc.], 1907, xxxvi, Orig., 415.

### MYCOBACTERIUM LACTICOLA δ FRIBURGENSE Korn

Obtained from butter by Korn.[1]

**Morphology.**—Very small rods three times as long as broad, very irregular and quite different from the tubercle bacillus. Often club-shaped, arranged in palisades and resem-

bling diphtheria bacilli. No branching. No motility. No spores. In milk cultures the organisms resemble tubercle bacilli.

**Stains** by ordinary anilin dyes. Gram-positive. Retains Ziehl-Neelsen's carbolfuchsin when treated with alcoholic solution of nitric acid. In pure culture resists decolorization three minutes in 10 per cent. alcoholic solution of nitric acid.

**Cultural Characters.**—No growth in *gelatin stabs* at room temperature. Growth on ordinary media at room temperature minimal. On *agar* negative. Optimum temperature 37° C.

On *glycerin agar* at 37° C. growth like that of the tubercle bacillus. It is pale yellowish, orange-red, dry, crumbly, and develops more rapidly than the tubercle bacillus, being visible on the third day. In older cultures the surface of the agar is covered with a thick, swollen, dry layer of bacteria which rises up over the water of condensation on the edge of the glass. The color is now a trifle deeper.

On *glycerin-horse-blood-serum* the growth is light orange, moist, glistening and raised. No film forms on the water of condensation which develops a milky-white turbidity.

On the surface of *glycerin peptone broth* the organisms grow as a delicate film which after a long time sinks to the bottom. The broth itself remains clear. No indol. No disagreeable odor. Reaction of broth alkaline.

On *potato* no development at room temperature. At 37° C. slight growth as a raised, dirty-yellow mass.

No gas from *glucose.*

The organism has considerable resistance to sunlight. Cultures preserved over six months in diffuse daylight are still viable.

**Pathogenic Action.**—Not virulent for mice. With pigeons and hens necrotic foci develop at the site of subcutaneous inoculation containing the characteristic organisms after two months. With guinea-pigs and rats intraperitoneal inoculation leads to the formation of tubercle-like lesions in the mesenteric glands in which the organisms can be demonstrated. No changes in other organs. Subcutaneous injection is followed by the formation of abscesses in which the organism can be found. The internal organs show no changes. In rabbits subcutaneous and intraperitoneal inoculation is followed by abscess formation at the site of inoculation while the internal organs show lesions which macroscopically cannot be differentiated from those of true tuberculosis. Microscopically the lung lesions contain giant-cells like those of true tubercles which they resemble closely. The nodules in the liver, however, are not so typical.

Korn's observations on animals were confirmed by Aujeszky,[2] who found that a frog injected intraperitoneally with 1 c.c. of an old milk culture died after eighteen days. At autopsy small "pseudotubercles" were found in the abdominal cavity.

1. Korn: Centralbl. f. Bakteriol. [etc.], 1900, xxvii, 481.
2. Aujeszky: Centralbl. f. Bakteriol. [etc.], 1907, xxxvi, 415.

### Moeller's Pseudoperlsucht Bacillus

An acid-fast and alcohol-fast bacillus found by Moeller in tuberculous nodules in cattle, differing from the tubercle bacillus in morphology, staining properties, and cultural reactions.

Microscopically the same length as the tubercle bacillus, but generally thicker. The organisms grow rapidly, appearing on artificial media in ten to twelve hours at 37° C. After twenty-four hours there is an abundant growth of colonies. At room temperature the growth is slower, but definite.

On *glycerin agar* the growth of pure culture is at first moist, later dry, dirty gray, membranous-like, and easily differentiated from the growth of the tubercle bacillus. Water of condensation remains clear.

Produces pseudotubercles in guinea-pigs.

Moeller: Centralbl. f. Bakteriol, [etc.], 1901, xxx, 513.

### Mycobacterium alluvialum (Kersten) Com.

Described by Kersten and isolated from soil at Berlin by the antiformin method of Uhlenhuth and Kersten.

**Morphology.**—Slender, fairly long, often curved rods, sometimes swollen at one end. Short forms also observed. Resembles the diphtheria bacillus. Branching present. Non-motile.

**Staining Reactions.**—Stains badly with methylene-blue, well by Gram and Ziehl-Neelsen,

the latter method revealing beautiful barred forms. Not decolorized by the usual acids and alcohol used for decolorization.

**Cultural Characters.**—Grows in two to six weeks on all media, best on ordinary beef serum and glycerin beef serum. Optimum temperature between 25° and 32° C.

*Gelatin Plates.*—Punctiform, later round, glistening yellow colonies. Under a magnification of 60 diameters, edges smooth, central portions more or less folded, colonies slightly raised with many branching, strongly reflecting, white winding striations. Both deep and superficial colonies the same. No liquefaction.

*Gelatin Slant.*—At first dirty-white expansion, glistening like fat, later yellow-brown.

*Agar* and *Glycerin Agar Slant.*—After six days a dirty-white granular layer, later made up of concentric wart-like yellow-brown masses the size of lentils.

*Serum and Glycerin Serum.*—Citron-yellow, homogeneous glistening soft expansion. Colonies crumbling, round, with irregular edges, citron-yellow, at first the size of pin-heads then becoming as large as lentils.

*Broth.*—At first a turbidity, later clearing. Sediment yellow grayish-red. No scum.

*Glycerin Potato.*—Thick, folded, granular, dirty-white expansion, later raised and bulbous. No pigment.

Indol formed in traces. Abundant $H_2S$. Moderate gas formation on sugar media.

*Non-pathogenic* to rabbits and guinea-pigs on subcutaneous inoculation of large doses, except for an occasional local abscess which breaks and heals spontaneously.

Kersten: Centralbl. f. Bakteriol. [etc.], 1909, li, Orig., 494.

### GENUS 2. CORYNEBACTERIUM Lehmann and Neumann

Slender, often slightly curved rods with tendency to club and pointed forms, branching cells reported in old cultures. Barred, uneven staining. *Not acid-fast. Gram-positive.* Non-motile. Aërobic (rarely anaërobic). No endospores. Some pathogenic species produce a powerful exotoxin. Characteristic snapping motion is exhibited when cells divide.

A. *Aërobes*

Type species *Corynebacterium diphtheriæ* (Klebs-Loeffler) Lehmann and Neumann.

" *Hofmanni*
" *xerosis* (Neisser and Kuschbert).
" *xerosis canis* (Graham-Smith).
" *acnes* (Gilchrist).
" *Hoagi* (Morse).
" *flavidus* (Morse).
" *Ruedigeri* (Mellon).
" *diphtheroides liquefaciens* (Graham-Smith).
" *coryzæ segmentosis* Cautley.
" *diphtheroides citreus* (Graham-Smith).
" *diphtheroides brevis* (Graham-Smith).
" *maculatum* (Graham-Smith).
" *auris* (Graham-Smith).
" *ceruminis* (Graham-Smith).
" *diphtheroides gallinarum* (Graham-Smith).
" *cuculi* (Graham-Smith).
" *Hodgkini* Bunting and Yates.
" *paralyticans* (Robertson, M'Rae, and Jeffery).
" *plumosum* (Fox).
" *enzymicum* (Mellon).
" *pyogenes* (Glage).

De Witt's motile gas-producing diphtheroid.

B. *Anaërobes*

*Corynebacterium lymphophilum* (Torrey).
Bacillus D of Adamson.

### CORYNEBACTERIUM DIPHTHERIÆ (Klebs-Loeffler) Lehmann and Neumann

First observed and described in 1883 by Klebs[1] from the membrane in diphtheria. Obtained in pure culture by Loeffler[2] the following year on solid media. Its relation to membranous croup was clearly established by the work of Loeffler and of Roux and Yersin[3] and since then has been universally confirmed. The organism also occurs in non-membranous inflammations of the throat, occasionally in wound infections, in puerperal infections, and somewhat rarely in lesions of the internal organs.

**Morphology.**—Slender irregular rods varying in length and breadth, both in the exudate and in artificial cultures. They vary in length from 1 to 6 $\mu$ and in width from 0.3 to 0.8 $\mu$ (Park and Williams[4]). They occur singly, in pairs and in short chains. They are often curved and show peculiar clubbed enlargements at the ends or in the middle. These club-shaped forms are characteristic as was originally pointed out by Loeffler. While often regarded as involution forms produced in old cultures, Neisser and Gins[5] have shown by staining with colloidal silver that club-shaped organisms occur in young six-hour cultures. These authors believe that while pseudodiphtheria bacilli may show spindle-shaped organisms, true button-like or club-like enlargements are found only in the diphtheria bacillus. In twenty-four-hour cultures the organism is very irregular, showing terminal club-shaped or spherical enlargements and long forms swollen in the middle. True diphtheria bacilli are apparently more irregular than the other members of this group like the Xerosis bacillus or Hoffman's bacillus.

The diphtheria bacillus rarely shows true branching as established by Kanthack,[6] Hill,[7] and Abbott and Gildersleeve.[8] This is regarded by some authors as evidence that it belongs to the higher bacteria. The organisms are sometimes arranged in groups or with two elements almost parallel or so disposed as to look like a T, an L, or a Y. They divide in a characteristic fashion by snapping. Hill thinks that the organism lies in a membrane which is bent or indented at the time of division, but does not break. In consequence the two young cells may retain a certain juxtaposition. Non-motile. No spores.

**Staining Reactions.**—The diphtheria bacillus stains by all the anilin dyes, but seems to have a special affinity for alkaline methylene-blue (Loeffler's). Stained by this dye it shows a peculiar segmentation into granules which makes it resemble a streptococcus. The granules may be brought out by double staining with carbolfuchsin and methylene-blue (Escherich[9]), appearing red in a blue background. Granules staining with methylene-blue are present in nearly all true diphtheria bacilli, but also in closely related organisms like the Xerosis bacillus (Ernst[10]). They are usually called Babes-Ernst bodies, and are no longer regarded as spores. They are more common in bacilli taken from the membrane than from cultures, more common in young cultures on Loeffler's serum than on agar, on gelatin or in broth. Granules may be demonstrated by Neisser's stain (acetic acid methylene-blue followed by Bismarck brown) and by those of Roux and Yersin, Loeffler, Falieres, and Ljubinsky among others. The diphtheria bacilli are always Gram-positive.

*Types of Diphtheria Bacilli.*—Following the disposition of the granules in the organisms Cobbet[11] divided them into 5 types. This work was followed by that of Wesbrook, Wilson, and McDaniel,[12] who made out 3 main groups in the diphtheria cultures examined in Minneapolis and a larger number of types. The same types have been found in other cities in America, not always in the same proportion as in Minneapolis. The following classification corresponds in its essentials to that of Wesbrook, Wilson, and McDaniel:

Group I. Granular forms showing distinct spherical, ovoid, or markedly rounded granules.

Group II. Barred forms showing darkly staining areas alternating with pale areas.

Group III. Solid color forms of various sizes and shapes.

Among the types established by Wilson, A, $A^1$, and $A^2$ represent the swollen bizarre forms often spoken of as involution forms, spherical, dumb-bell, Indian club, etc. They may be 1 to 2 $\mu$ in thickness and 3 to 6 $\mu$ in length. Occasionally they are even 3 $\mu$ in diameter. A is the granular type, $A^1$ the barred type, and $A^2$ the solid staining variety. B, $B^1$, and $B^2$ represent involution or attenuated forms. They are long and slender, about 0.5 $\mu$ in thickness and 3 to 7 $\mu$ in length. B is granular, $B^1$ segmented, and $B^2$ solid staining. C, $C^1$, and $C^2$ are the size usually seen in diphtheria, 0.5 to 1 $\mu$ in thickness and 3 to 6 $\mu$ in length. C shows the characteristic polar granules, $C^1$ is barred, and $C^2$ solid staining. D, $D^1$, and $D^2$ are smaller than the C types, about 0.75 to 1 $\mu$ in thickness and 2 to 3 $\mu$ in length. D shows polar granules, $D^1$ is barred, and $D^2$ solid staining, but frequently wedge-shaped or pointed at the ends. E, $E^1$, and $E^2$ are smaller than D; 0.5 to 0.75 $\mu$ in width by about 1.5 $\mu$ in length. E shows polar granules, $E^1$ is barred, and $E^2$ is solid staining and often pointed. F represents the delicate slender forms, 0.25 to 0.5 $\mu$ in breadth by 1 to 2 $\mu$ in length. F shows metachromatic granules and $F^1$ is solid staining. G represents the very short forms, 0.5 to 0.75 $\mu$ in thickness by 1 to 1.5 $\mu$ in length. G is granular and $G^1$ solid staining.

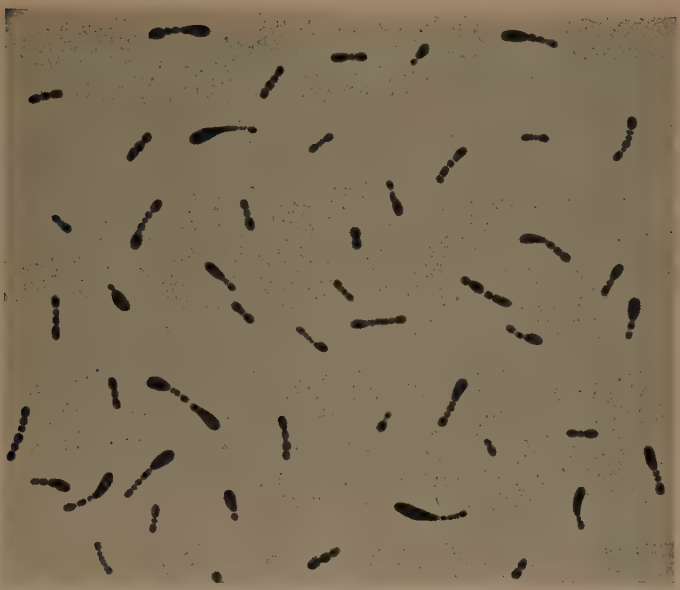

Fig. 12.—*Diphtheria bacillus*, Type A, from forty-eight-hour culture on Loeffler's blood-serum, stained with methylene-blue.

Fig. 13.—*Diphtheria bacillus*, Type A, $A^1$, $A^2$, from twenty-hour culture on Loeffler's blood-serum, stained with methylene-blue.

Fig. 14.—*Diphtheria bacillus*, Types B, $B^1$, $B^2$.

Fig. 15.—*Diphtheria bacillus*, Types C, $C^1$, from Loeffler's blood-serum, stained with methylene-blue.

Fig. 16.—*Diphtheria bacillus*, Type $C^2$, from twenty-hour culture on Loeffler's blood-serum, stained with methylene-blue.

Fig. 17.—*Diphtheria bacillus*, Type D, from Loeffler's blood-serum, stained with methylene-blue.

**Cultivation.**—Diphtheria bacilli can be cultivated without great difficulty on the ordinary laboratory media. They are usually associated with other organisms in the throat, however, and may be isolated most easily on *Loeffler's blood-serum.* On this media the organisms grow rapidly, appearing in twelve to fifteen hours as small discrete raised colonies, pearl gray, whitish-gray, or yellowish-gray in color. A visible growth sometimes appears in six hours at 37° C. By the end of twenty-four hours the colonies are much larger, smaller than staphylococcus colonies and larger than streptococcus colonies. Older colonies are larger, less glistening, with irregular edges and sometimes concentric rings of growth or fine radial striations. The colonies may remain isolated or may coalesce to form a moist, thin, grayish-white or grayish-yellow expansion which is usually limited to the line of inoculation. Winslow and Hill[13] found that some cultures have a yellowish color on Loeffler's serum, others are pale red and quite old cultures dark brown or black. Similar pigmented strains have been noted by v. Przewoski[14] and by Neisser and Gins.

The growth characters on Koch's coagulated serum are much the same as on Loeffler's serum.

*Agar.*—On ordinary agar the growth is moderate, colonies being visible after twenty-four hours and appearing under low magnification as small deposits with an irregular, often a feathery contour. Centers yellowish, edges white, surface uneven, feathery and coarsely granular. After several days' growth the colonies may have a diameter of 1 mm. or more. They often show radial striations and concentric rings. Daughter colonies may also appear. On slant agar pure cultures form a delicate grayish-white vapor-like or hazy expansion. On slightly alkaline agar or on glycerin agar the diphtheria bacillus grows well after cultivation for several generations on blood-serum. Freshly isolated strains grow very slowly, however. Characteristic colonies appear in twenty-four to forty-eight hours, round, regular; under low power granular, denser in the center, with thin, somewhat jagged, irregular fringe-like margins in some cases, entire edges in other cases. The deep colonies are punctiform; under low power round or oval disks.

According to Park and Williams the growth of the diphtheria bacillus is more luxuriant, but less characteristic upon a mixture of 2 parts of 1.5 per cent. nutrient agar and 1 part sterile ascitic fluid.

*Blood Agar.*—Mandelbaum and Heinemann[15] report that the diphtheria bacillus produces white colonies with brown-yellow zones on glycerin agar containing a few drops of human blood, while pseudodiphtheria bacilli produce red colonies with brownish-red zones. Neisser and Gins have shown that rabbit blood serves the same purpose and may be mixed with the agar. On this medium the diphtheria bacillus produces greenish colonies with clear zones about them, pseudodiphtheria bacilli white colonies without zones.

*Gelatin.*—Colonies are like those on agar; under low power pale brown and sharply contoured. After several days new areas of growth may appear on the edges of the colonies which now are mulberry-like. In a gelatin stab a whitish growth appears along the line of inoculation with a firm granular expansion on the surface. No liquefaction.

*Potato.*—Growth scanty, thin, whitish.

*Broth.*—In neutral alkaline broth many strains of the diphtheria bacillus grow readily, producing a fine granular deposit on the bottom with a dust-like growth along the walls of the container. Other strains produce a diffuse cloudiness with a fragile film on the surface which sinks to the bottom on shaking. In old cultures the organisms have a more characteristic growth, producing regularly a fragile pellicle on the surface with clear broth below. According to Robinson and Meader[16] the addition of a piece of sterile guinea-pig liver to the broth favors the pellicle formation.

In broth containing muscle sugar the reaction becomes acid, as first shown by Smith.[17] In sugar-free broth the reaction becomes alkaline. Neisser and Gins have found that every typical strain of the diphtheria bacillus produced acid in broth not rendered sugar-free.

*Ascites or Serum Broth.*—According to Park and Williams the diphtheria bacillus grows well in nutrient broth to which 25 per cent. ascitic fluid or serum is added, producing a slight pellicle in twenty-four to forty-eight hours.

*Milk.*—Growth rapid both at 20° C. and at 37° C. without change in reaction.

*Carbohydrates.*—The diphtheria bacillus produces acid from the monosaccharids, dextrose, galactose, levulose, but not as a rule from other carbohydrates. Exceptions have been noted, saccharose being attacked according to Martin,[18] maltose according to Knapp[19] and Graham-Smith,[20] lactose according to Graham-Smith, dextrin according to Knapp.

In the Smith fermentation tube containing dextrose broth the organism produces a distinct turbidity in the bulb with a fragile pellicle and a well-marked turbidity in the closed arm.

The acid reaction persists for a time, but later the reaction becomes neutral or alkaline.

Other media valuable for the cultivation of this organism are Deycke's alkali-albuminate agar, pepsin trypsin agar, glycerin ascites agar, Lubenau's egg-yolk-glucose agar, Conradi and Troch's telluride agar, and Rankin's potassium sulphocyanide and neutral-red sheep serum.

Optimum temperature 35° to 37° C. Growth from 20° to 41° C. Growth best on

Diphtheria bacillus, Type C, mixed with staphylococci, from Loeffler's serum, stained with methylene-blue.

Diphtheria bacillus, Type C, stained by Lubinski's method.

alkaline media, an alkalinity of 6 to 8 c.c. normal sodium hydrate to 1000 c.c. being most favorable. An alkalinity of 30 c.c. normal sodium hydrate to the liter hinders the growth. Growth ceases at an acidity of 12.5 c.c. to the liter.

**Resistance.**—Destroyed by ten minutes exposure to 60° C., five minutes exposure to 70° C., and almost instantly by boiling. When in a fine spray destroyed in diffuse daylight in twenty-four to forty-eight hours (Kirstein). Survives for a long time in a diphtheria membrane—for fourteen weeks (Loeffler), for seventeen weeks (Park and Beebe), for five months in the dark (Roux). Dried on silk threads survives eighty-six days (Abel). Destroyed by rapid drying over sulphuric acid in forty-eight hours (Reyes). In dried agar cultures still alive after twenty days (Hill).

Destroyed rapidly by disinfectants, in 1 per cent. carbolic acid in ten minutes (Smith and Somerville), in 1 per cent. sublimate solution in one minute, in 1 per cent. beta-naphthol in ten minutes, and in 1 per cent. tribrom-beta-naphthol in one minute (Bechhold), in 30 to 60 per cent. alcohol in five minutes when dry and in one minute when moist (Russ).

Resistant to low temperatures, surviving exposure to the temperature of liquid air (−190° C.) for ten days (McFadyean and Rowland).

In cultures the diphtheria bacillus survives long periods; in dried agar twenty days (Hill); on serum cultures one year (Heim); on agar cultures one hundred and sixty to one hundred and seventy days (Abel), seven months (Park), eighteen months (Klein).

In milk the organisms may survive for a long period, in raw buttermilk only one to seven days.

**Pathogenic Action.**—The diphtheria bacillus is pathogenic to a large number of small animals, such as guinea-pigs, rabbits, chickens, pigeons, small birds, cats, dogs, and puppies, but has little virulence for rats and mice. It is also pathogenic to large animals such as cattle and horses. The pathogenic action is due almost entirely to the powerful toxin secreted by the organisms since diphtheria bacilli washed free from toxin are almost without action on animals. The lesions have been studied most carefully in the guinea-pig, chiefly because this animal has come into general use for the testing of diphtheria toxin and antitoxin. In these animals minute quantities of virulent cultures injected subcutaneously produce symptoms of illness in twelve to twenty-four hours, ruffled hair, lack of appetite, unwillingness to move, and sensitiveness to pressure over the injected area, which is swollen and infiltrated. With cultures of high virulence the animals die in two to four days from dyspnea. At autopsy an extensive grayish-red gelatinous exudate is found in the entire abdominal wall, surrounded by a zone of injected blood-vessels, while at the site of inoculation grayish necrotic masses are present which microscopically reveal diphtheria bacilli. The neighboring lymphatic glands are swollen and hemorrhagic, the lymphatic vessels enlarged. A serous or bloody exudate is occasionally found in the abdominal cavity. The internal organs are hyperemic, particularly the adrenals which are enlarged, reddish-yellow or dark red. The intestines are injected and frequently minute ulcers with hemorrhages appear in the mucous membranes of the stomach and large intestine. Rosenau and Anderson[21] find that 60 per cent. of the guinea-pigs dead of diphtheria infection or intoxication show ulcers in the stomach like round ulcers in man. A serous or bloody exudate also occurs in the chest, sometimes as much as 10 c.c. in quantity (Neisser and Gins). The lungs may show areas of atelectasis, areas of hemorrhage, or at times true pneumonia.

Microscopically the tissues at the site of inoculation show necrosis with organisms lying free in the tissue spaces or taken up by the leukocytes, only at or near the site of injection. The necrosis may also involve the leukocytes which have wandered into the area. The internal organs show areas of fatty and hyaline degeneration and necrotic foci, especially in the liver and heart muscle (Pratt).

While diphtheria bacilli are present at the site of inoculation at times in great abundance, they are found in the internal organs in only a small percentage of cases. Thus Wright[22] found them in the liver in 12 per cent. of his animals, in the spleen in 9.8 per cent., in the kidneys in 2.6 per cent. They are more common in the blood, in 45 per cent. according to Wright.

With less virulent cultures the animals survive longer periods, the same exudate developing at the site of inoculation. This may slough and heal or persist till the death of the animal from marasmus. At autopsy essentially the same lesions are found as in the acute cases. Occasionally the animals develop a paralysis which closely resembles the postdiphtheritic paralysis seen in man. Guinea-pigs also succumb to intraperitoneal injection with hemorrhagic lesions in the peritoneum.

Rabbits are easily infected both by subcutaneous inoculation and by intravenous injection (Loeffler, Roux and Yersin, Méten[23]).

In nearly all animals which are susceptible to the diphtheria bacillus chronic intoxication may develop from small doses of very virulent cultures or large doses of less virulent organisms. This has been especially studied in pigeons, hens, rabbits, and guinea-pigs by Loeffler, in pigeons by Spronck[24], in rabbits by Roux and Yersin and by Babes.[25]

While these lesions correspond in general to the human lesions, the organisms when implanted on the healthy mucosa of animals usually fail to survive. If this mucosa be

scarified or injured, however, and then exposed to virulent diphtheria bacilli lesions develop which are like the membranes in man. Thus membranes have been produced in this way on the pharyngeal mucosa of apes, fowls, pigeons, and on the trachea and conjunctiva of rabbits. In rabbits inoculated directly through the opened trachea pseudomembranes are produced much like those found in membranous croup (Loeffler, Roux and Yersin, Hencke[26]) If tracheotomy be performed and the mucous membrane kept from injury the implantation of the diphtheria bacillus on it results only in small inflamed areas, although fatal infections may develop by way of the lungs (Flexner and Anderson[27]). With inoculation in the conjunctiva of rabbits thick exudates develop in twenty-four hours which are usually fibrinous (Babes).

**Diphtheria Toxin.**—The diphtheria bacillus owes its ability to produce disease in man and animals to a true extracellular soluble toxin suspected by Loeffler, but first obtained by Roux and Yersin and carefully studied by them. This toxic substance is produced by the diphtheria bacillus in cultures, preferably in broth, and minute doses are fatal to the same animals as are susceptible to the action of the bacillus. The effect of the toxin has been most carefully studied in guinea-pigs. After an incubation period of eight to twelve hours the animals show signs of illness resembling those seen in animals inoculated with living organisms. With proper dosage they may succumb to the intoxication in three to four days, but with smaller doses may survive five to six weeks. In animals acutely poisoned there is the usual edematous infiltration at the site of inoculation, leading to a slough if the animals survive long enough. The internal organs, especially the adrenals, present much the same picture as is seen in inoculations with living organisms. The diphtheria toxin is a substance of unusual strength, $\frac{1}{100}$ to $\frac{1}{1000}$ c.c. of a suitable broth culture often killing guinea-pigs in three to four days. It is thermolabile, being rendered inert in thirty minutes at a temperature of 58° to 60° C. (Roux and Yersin). Owing to the fact that the antitoxin to it, originally discovered by von Behring,[28] has proved to have amazing therapeutic properties various attempts have been made to obtain potent toxins. The usual method at present employed is to cultivate virulent organisms in sugar-free broth for a number of days, the maximum toxin production being reached on about the fifth to sixth day in some strains and after three to four weeks in other strains. It is noteworthy that toxin production takes place only where the bacillus grows as a film on the surface of broth. Media therefore which enable the organism to grow on the surface favor toxin production. Nothing is definitely established about the chemical composition of the diphtheria toxin. It has been regarded as an enzyme by Loeffler, as a toxalbumin by Brieger and Fraenkel. Preparations not giving protein reactions have been obtained by Brieger and Boer,[29] while Guinochet[30] has found it in protein-free urine. It is usually regarded as a product of the living bacterial cells and not produced with the death of the cell. It may be dried without losing its potency, dried toxin resisting 70° C. for some time and 100° C. for twenty minutes. It is destroyed by organic acids, but is relatively resistant to inorganic acids, 1 per cent. HCl, 1 per cent. carbolic, to benzol derivatives, iodoform, chloroform, etc.

**Virulence.**—The virulence of the diphtheria bacillus is due almost exclusively to the toxin it produces, and it is doubtful if non-toxic strains have any effect on animals. In consequence we estimate the virulence of this organism by determining the amount of toxin present in cultures. A strain is regarded as fully virulent if small quantities kill a guinea-pig of 250 gram weight in four days with the usual lesions. The actual amount of the culture required to bring about this reaction is subject to great variation, and depends on a number of factors. Thus certain strains may be good toxin-producers immediately after isolation, others may become so after laboratory cultivation, and still others may lose their ability to produce toxin after prolonged growth under artificial conditions.

**Diphtheria Antitoxin.**—The antitoxin for the poisons of the diphtheria bacillus, discovered by von Behring, is obtained usually from horses by their protracted immunization with diphtheria toxin. The initial doses of toxin are frequently mixed with some substance like iodin trichlorid or tetrachlorid. In Roux's method a mixture of toxin and antitoxin is first given followed by the pure toxin, or antitoxin given first followed by toxin. The diphtheria toxins are standardized by the inoculation of guinea-pigs of uniform weight, 250 grams; that amount which will kill the animal in four days being called the minimum lethal dose (M. L. D.). The toxin is administered to the horses at regular intervals and the serum drawn off by venesection after the lapse of sufficient periods of time for the elaboration of patent antitoxins.

The standardization of diphtheria antitoxin for therapeutic purposes is now carried out by the methods originally proposed by Ehrlich. A unit of antitoxin theoretically is the amount which will completely neutralize 100 minimum fatal doses of toxin for a 250-gram guinea-pig. Since toxin deteriorates rapidly the antitoxins are now standardized against standard antitoxin distributed from a central laboratory such as the Institute f. experimentelle Therapie in Frankfort, Germany, or the Hygienic Laboratory of the Public Health Service in Washington.[31] The standard antitoxin is sent out at regular intervals, each bottle being marked with the number of units of antitoxin in each cubic centimeter. The toxin to be tested is standardized by mixing it with one antitoxic unit, represented by various dilutions

of the test antitoxin. The mixtures are allowed to stand fifteen minutes for the union of the toxin and antitoxin and tested upon 250-gram guinea-pigs. The L+ dose of the toxin is thus determined, representing the quantity of toxin which neutralizes an antitoxic unit and contains beyond this enough toxin to kill a guinea-pig of 250 gram weight in four to five days with characteristic lesions. After the determination of the L+ dose of toxin varying amounts of the unknown antitoxin are mixed with this quantity and the mixtures injected into guinea-pigs of standard weight. The unit of antitoxin is the amount which will exactly neutralize this L+ dose.

Diphtheria antitoxin contains 3000 to 20,000 units per cubic centimeter and this amount is usually regarded as a therapeutic dose. For prophylactic immunization a dosage of 500 units is generally employed.

Diphtheria antitoxin may be concentrated by precipitation with an equal volume of saturated ammonium sulphate solution by Gibson's method. About four-fifths of the original quantity of antitoxin is obtained in a concentration one-fifth to one-seventh as great as the original horse serum. The strength of the concentrated product is determined by the method followed for the standardization of whole horse antitoxic serum.

*The Schick Test.*—The Schick reaction is carried out by the administration of small quantities of standardized diphtheria toxin, usually $\frac{1}{50}$ of the minimum lethal dose for a guinea-pig. The dilutions are so made that this quantity is contained in 0.1 c.c. of solution. A *positive reaction* consists of a slight infiltration of the skin and a red areola 1 to 2 cm. in diameter about it which appears in twenty-four to thirty-six hours in individuals who do not have sufficient antitoxin in their blood to neutralize this small quantity of toxin. When the blood has less than $\frac{1}{50}$ unit of antitoxin per c.c. a positive reaction is usually given. A *negative reaction* or a failure to react to the injected toxin indicates the presence of sufficient antitoxin in the blood to neutralize the toxin given. This amount is usually $\frac{1}{50}$ unit of antitoxin per cubic centimeter of blood or more than this amount.

**Distribution.**—The diphtheria bacillus is present in the majority of inflammations of the throat in which a membrane is formed (usually called a pseudomembrane or false membrane), and in many milder infections where no membrane appears. It is also present in nasal diphtheria, which may occur as a primary disease or as an extension from the pharynx, and occasionally in bronchopneumonia occurring in the course of the infection (Loeffler, Strelitz[32]). It is rarely present in inflammation of the mucous membrane in other parts of the body. Thus it may cause a membranous inflammation of the ocular conjunctiva, and of certain areas of the genitalia, as the vulva. It is an important agent in wound infections, and is frequently isolated from infected gun-shot injuries. In both the natural disease and in artificial infections in animals the organisms are usually limited to the site of the local inflammation. They may get into the blood-stream, but do not multiply in it. Metastatic inflammation due to the diphtheria bacillus may occur in the mucous membrane of the stomach, in the heart valves, and in the pericardium.


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19. Knapp: J. Med. Research, 1904, xii (n. s. vii), 475.
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21. Rosenau and Anderson: J. Infect. Dis., 1907, iv, 1.
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23. Méten: Ann. d. l'Inst. Pasteur, 1898, xii, 596.
24. Spronck: Ann. d. l'Inst. Pasteur, 1895, ix, 758.

25. Babes: Ztschr. f. Hyg., 1895, xx, 412.
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### Pseudodiphtheria bacilli or diphtheroids

In addition to the true diphtheria bacilli and the non-virulent strains which have the same morphology and cultural reactions, a large number of organisms have been isolated from time to time which are grouped together as pseudodiphtheria bacilli or diphtheroids. Organisms called diphtheroids show the greatest diversity in morphological features, staining reactions, and cultural properties. Some of them have the morphology of the Klebs-Loeff-

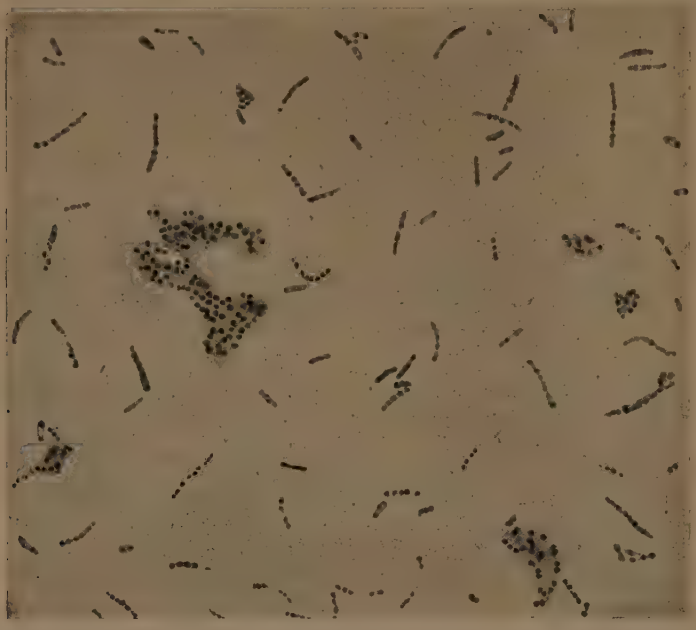

Fig. 18.—*Pseudodiphtheria bacillus* from intestinal contents, eight-day culture on blood-serum, stained with methylene-blue.

ler organism, but differ from it in cultural reactions. Others are non-motile pleomorphic bacilli which resemble the diphtheria bacillus only superficially, showing irregular staining by Loeffler's methylene-blue or Neisser's stain. Still others are acid-fast or partially acid-fast. The majority grow well on ordinary media under aërobic conditions, a few growing only under partial tension or as obligate anaërobes. They are sometimes pathogenic to small animals, but the lesions are not like the lesions produced by the diphtheria bacillus. None of them produce the true diphtheria toxin.

The diphtheroids are widely distributed. They have been found in the air by Sudeck,[1] Neumann,[2] Wade and Harris,[3] Torrey[4] and Mellon,[5] in water by McNaught, in milk by McCambell and Graham-Smith,[6] and by Bergey.[7] They occur especially on the surface of the body, in normal and pathological lymph-glands and in various diseased organs to which they have gained access either from the skin or mucous membranes. They are frequently encountered

in the upper respiratory tract, on the skin of the genitalia, and about the urethra. Certain of these isolations deserve special mention. Thus diphtheroids have been isolated by Davis[8] from 12 cases of scarlatinal otitis media, by Williams[9] in 8 cases out of 62 examined, and by Forbes[10] in 32 out of 40 cultures from similar cases.

Two varieties of pseudodiphtheria bacilli were found in suppurative processes by Hamilton,[11] especially in the pus of postscarlatinal otitis media. They were found so frequently in this disease that they may play an important part in its causation. No less than 72 per cent. of 43 cases of acute scarlatinal otitis media gave cultures of these bacilli and 20 per cent. gave pure cultures. Only 21 per cent. of cases of acute non-scarlatinal otitis gave the same bacilli.

Group 1.—Ferments saccharose, but not maltose, is seldom virulent for guinea-pigs, and is agglutinated and killed by the serum of rabbits immunized against one member of the group. May or may not be Gram-positive.

Group 2.—Ferments maltose, but not saccharose, is more often virulent for guinea-pigs and is not affected by serum of rabbits immunized against Group 2. Neither group affected by serum from "Ruediger bacillus."

Diphtheroids have been obtained from enlarged glands and from certain organs in a variety of diseased conditions. They have been cultivated from the spleen in splenic anemia

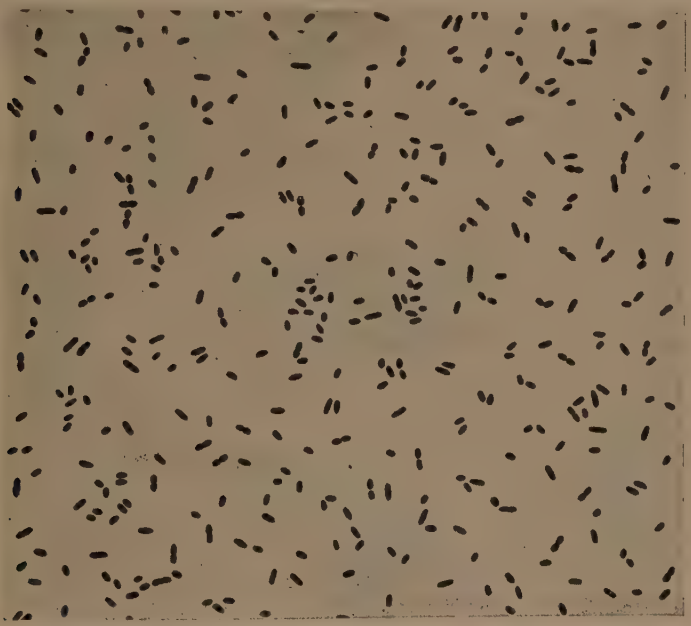

Fig. 19.—*Pseudodiphtheria bacillus* from otitis media, blood agar plate twenty-four hours old, stained by Gram.

by Lanford,[12] and by Bunting, Yates and Kristjansen,[13] from the lymphatic glands in a case of acute leukemia by Steele,[14] and by Simon and Judd,[15] from chronic leukemia and pseudoleukemia by Bunting and Yates,[16] from enlarged glands in tuberculosis and mediastinal tumor by Fox,[17] from diseased lymphatic glands and from those approaching a normal condition by Bloomfield.[18] They were isolated from 8 cases of erythema nodosum by Rosenow,[19] and regarded by him as the etiological factor in the disease. These strains were described as Gram-staining, pleomorphic, non-motile, non-spore-forming bacilli, producing small round colonies in dextrose agar, small gray or yellowish non-hemolyzing colonies on blood agar and having a wide range of fermentative activity. They were pathogenic to rabbits in very large doses, 4 to 60 c.c.

Diphtheroids growing only under anaërobic conditions were found in the urine by Dick[20] in chronic non-suppurative nephritis. Some of them were Gram-positive, others Gram-negative. Anaërobic and partial tension diphtheroids were also obtained by Bloomfield.

We will describe those diphtheroids which seem to be distinct species. No satisfactory classification has thus far been devised. Morse[21] has suggested the following method of grouping them.

**Morse's Classification of Diphtheroids.**—*Group A.*—Medium-sized bacilli showing solid barred and wedge forms with abundant but small and imperfect granules. On serum it produces a very heavy confluent glistening growth with a characteristic salmon-pink color. It ferments dextrose and saccharose, but not maltose or glycerin.

Properly named *Bacillus Hoagii.*

*Group B.*—Organisms larger than those of Group A and thick forms with clear-cut bars predominate. Neisser's granules are very large and irregular. The growth on serum is heavy and varies in color from white to yellow. It is often noticeably dry and granular. Dextrose is always fermented, maltose and glycerin usually, but not saccharose.

Named *Bacillus flavidus.*

*Group C.*—Organisms resemble Group B. Differentiated by slow, scanty, colorless or white growth. They always acidify dextrose and usually both maltose and saccharose. Properly named *Bacillus xerosis.*

*Group D.*—Small thick straight bacilli, often barred and wedge-shaped, showing no granules. Growth on serum may be scanty or abundant and is white or yellow-white in color. The organisms do not act on dextrose, maltose, glycerin, or saccharose.

Properly called *Bacillus Hofmanni.*

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11. Hamilton: J. Infect. Dis., 1907, iv, 326.
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21. Morse: J. Infect. Dis., 1912, xi, 253.

### CORYNEBACTERIUM HOFMANNI

Originally observed by Loeffler[1] and described as a pseudodiphtheria bacillus. Extensively investigated by Hofmann.[2] Usually called Hofmann's bacillus. Frequently found vegetating on the normal mucous membrane of the nose and throat.

**Morphology.**—Short plump organisms, more uniform in size than the diphtheria bacillus. They may be barred and wedge-shaped, but are free from granules. They usually measure 0.3 to 0.5 $\mu$ in thickness by 0.8 to 1.5 $\mu$ in length (Bergey[3]).

**Stained** unevenly by Neisser's stain or alkaline methylene-blue. Gram-positive.

**Cultivated** without great difficulty on ordinary media, best at 37° C.

*Agar* colonies small, homogeneous, opaque, smooth, grayish to cream-colored, with entire edges. Moist, smooth, cream-colored growth on agar slants.

*Gelatin* colonies small, homogeneous, grayish or cream-colored. Slight surface growth in gelatin stabs, little growth along the line of inoculation. No liquefaction of gelatin.

*Blood-serum* colonies small, pearl-gray to gray-white, not unlike those of the diphtheria bacillus. The colonies may coalesce and produce a growth which is somewhat thicker than that of the Klebs-Loeffler organism. The blood-serum growth may also be scanty and at times yellowish colonies may be seen (Morse[4]).

No change in *milk*. Delicate cream-white growth in *potato*. Acids not formed from any carbohydrates, dextrose, maltose, saccharose, lactose, glycerin, etc.

Nitrates reduced to nitrites. Indol not produced.

Non-pathogenic.

1. Loeffler: Centralbl. f. Bakteriol. [etc.], 1887, ii, 105.
2. Hofmann: Wien. med. Wchnschr., 1888, xxxviii, 66; 107.
3. Bergey: Manual of Determinative Bacteriology. 1923, p. 385.
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### CORYNEBACTERIUM XEROSIS (Neisser and Kuschbert)

Described as the xerosis bacillus by Neisser and Kuschbert[1] from a peculiar condition of the conjunctiva known as xerosis. Also present in the normal conjunctiva and may have no relation to the disease in which they were originally found (Park and Williams[2]). They were isolated by Graham-Smith[3] from 9 healthy conjunctivas out of 10 examined.

**Morphology.**—Rather thin, fairly long, curved bacilli on blood-serum, slightly clubbed and segmented. They usually show 2 to 4 segments. Polar bodies are rare in fresh cultures, but subcultures may show them large and well developed (Graham-Smith). Frequently arranged in small groups. The organisms are broader on agar than on serum, well segmented, and usually without polar bodies. Short on potato, like Hofmann's bacillus, but tending to produce irregular forms. Non-motile.

**Stained** easily by the usual anilin dyes, best with methylene-blue or Neisser's stain. Gram-positive.

**Cultivated** without especial difficulty on ordinary media, best at 37° C. The original cultures tend to grow slowly, but subcultures grow more rapidly.

*Agar* colonies minute, raised, round, smooth, almost transparent in forty-eight hours. Very little increase in size even after several days' incubation. In *agar stabs* very minute colonies develop along the line of puncture with a small and almost transparent surface growth. Thin grayish limited growth on *agar slants*.

On *alkaline potato-agar* small, round, smooth, gray, dome-shaped colonies appear in forty-eight hours (Graham-Smith). Smaller but similar colonies on *acid potato agar*.

*Blood-serum* colonies resemble those of the diphtheria bacillus, but are usually very minute, in fresh cultures appearing only after forty-eight hours (Graham-Smith). They tend to adhere to the medium and have irregular edges after seventy-two hours.

*Gelatin* colonies very minute, round, and transparent. Many strains do not grow on gelatin. Occasionally very minute colonies in *gelatin stabs*.

Little if any growth on *potato*. No change in *litmus milk*. Faint granular sediment in *broth*.

Nitrates not reduced to nitrites. Indol not formed.

**Carbohydrates.**—Knapp[4] reports that the xerosis bacillus produces acid from dextrose, levulose, galactose, maltose and saccharose, the tests being made in Hiss's serum water medium with 1 per cent. agar.

No acid from other carbohydrates, including dextrin. Graham-Smith states that some cultures of this organism do not change the reaction in glucose broth.

Non-pathogenic.

1. Kuschbert: Deutsche med. Wchnschr., 1884, x, 321; 341.
2. Park and Williams: Pathogenic microörganisms. 1920, p. 358.
3. Graham-Smith: J. Hyg., 1904, iv, 258.
4. Knapp: J. Med. Research, 1904, xii (n. s. vii), 475.

### CORYNEBACTERIUM XEROSIS CANIS (Graham-Smith)

Obtained by Graham-Smith from the conjunctival sacs of three dogs.

On serum the colonies only make their appearance after two to three days' growth, though in later subcultures the growth is a little more rapid. After four to five days' growth the colonies are of large size. Except for their size the colonies are indistinguishable in appearance from those of the diphtheria bacillus, but tend to adhere to the medium. The organisms are long, curved, and stain well, showing well-differentiated, short, dark segments,

separated by narrow light bands crossing the bacillus transversely. Clubbing in some specimens is well marked. These organisms resemble closely the pseudodiphtheria type of Hofmann's bacillus. By Neisser's method variously shaped polar bodies are seen in large numbers in each bacillus. Some are large and round, others elongated transversely across the bacillus, and many are exceedingly small. The organisms are non-motile, and stain well by Gram's method. On agar after two or three days' growth small grayish colonies appear with irregular edges, and darker centers. On agar stab cultures the surface growth is small and almost transparent, but in the depth a few medium-sized round colonies develop. On alkaline potato agar after two or three days' growth medium-sized, whitish, opaque, dome-shaped colonies are seen, with a granular surface and very irregular margins. The organisms are oval and broad, but show well-marked segments. On acid potato agar the growth is similar. On gelatin no growth was obtained. No visible growth occurs in potato. Broth after forty-eight hours remains clear, but small granules are found at the bottom of the tube, which when shaken up float in lines as if held in position by invisible threads. The growth in glucose broth is similar, and its reaction after forty-eight hours is neutral. The organism is non-pathogenic.

Graham-Smith: J. Hyg., 1904, iv, 258.

### CORYNEBACTERIUM ACNES (Gilchrist)

Originally observed by Unna[1] in smears from comedones and first cultivated by Sabouraud[2] on acid glycerin agar. Cultivated from acne nodules

Fig. 20.—*Acne bacillus* from twenty-four-hour culture on agar, stained with safranin.

by Gilchrist[3] and named *Bacillus acne.* Gilchrist further found that the organism was agglutinated by the blood of patients with severe acne, and that it was pathogenic for small animals. Obtained first by anaërobic methods by Hallé and Civatte,[4] in deep shake tubes of glucose agar. The most successful results are those of Ketron and Burky,[5] who paid particular attention to the hydrogen-ion concentration of the media employed. Present usually in hair follicles and sweat glands.

**Morphology.**—Short thick bacilli in smears from the pus of the acne lesions, longer and thicker in cultures (Gilchrist). In smears from the lesions they are often arranged in groups. Branching forms are distinctly seen in old cultures on glycerin agar and blood-serum and in

broth, but rarely in the pus. In cultures a week old the organisms are about 1 $\mu$ wide and 2 to 5 $\mu$ long or even longer. They are arranged singly, in pairs or in groups. Some of the pairs are distinctly coccoid. The elongated forms are slightly curved as a rule and show some irregularity in outline. Old cultures show involution forms. According to Sabouraud the organisms are encapsulated, but capsules could not be demonstrated by Ketron and Burky. Non-motile.

**Staining Reactions.**—The organisms stain irregularly, especially with methylene-blue. Gram-positive.

**Methods of Isolation.**—Best growth in an acid medium with a hydrogen-ion concentration between 6.2 and 6.8. Multiplication slow and most luxuriant under anaërobic conditions. Growth under aërobic conditions possible if large quantities of material are transplanted. Enriching substances like dextrose or glycerin must be added to the media to obtain good cultures.

**Cultural Characters.**—*Meat Infusion Agar*, pH 6.2 to 6.8. Scanty growth without pigmentation (Ketron and Burky).

*Meat Infusion Agar*, pH 6.2 to 6.8 containing 1 per cent. dextrose or 2 per cent. glycerin. Growth appears under anaërobic conditions in one to two days and is luxuriant in one to two weeks. The entire inoculated area may be covered with a smooth, moist expansion, or ridges may develop with bead-like projections where the seeding is thickest (Ketron and Burky). The growth is raised and luxuriant, but does not spread beyond the margin of inoculation. It is soft, moist, non-viscous, and easily emulsified in broth or salt solution. The color varies from a faint pinkish to a deep pink or red. Under aërobic conditions growth is slower and is not usually apparent for four to five days. It gradually increases in size for three to four weeks. It appears as a heaped-up putty-like mass, smooth, round, or oval, 4 mm. in diameter and 2 to 3 mm. in depth. In old cultures the color is grayish-brown when growth is good, but the pigmentation is often lacking with poor growth. In Gilchrist's isolations the color was almost black in old glycerin agar cultures.

*Ascitic Fluid and Rabbit's Blood Agar.*—Development excellent, like that on dextrose or glycerin agar.

*Extract Agar.*—Growth usually absent. With 1 per cent. dextrose added a scanty aërobic or anaërobic development may occur.

*Agar Plates.*—The acne bacillus does not grow well in plates (Ketron and Burky). Occasionally fine pin-point colonies may appear in the depth in dextrose infusion agar or in 5 per cent. rabbit blood agar, incubated under anaërobic conditions. Rarely large surface colonies, 2 mm. in size and well pigmented, may appear with certain strains.

*Deep 1 per cent. Dextrose Infusion Agar Shake Cultures.*—Characteristic development. The colonies appear in two or three days in a narrow band one or more centimeters below the surface of the agar (Ketron and Burky). The band is sharply defined above and below and usually about 0.5 cm. wide. In older cultures the colonies may develop in the deeper portions of the agar all the way to the bottom or the lower mass of colonies may be separated from the upper growth by a narrow growth-free area 0.25 to 0.5 cm. in height. The colonies vary in size from pin-points to 2 mm. and are usually compound and resemble 2 convex disks intersecting each other at right angles. They are usually grayish in color, but occasionally pinkish or brownish. The larger colonies of the acne bacillus may be larger than staphylococcus colonies. The addition of ascites fluid to the medium increases the rapidity of growth.

*Fisk and Burky's hormone broth* containing 1 per cent. dextrose or 2 per cent. glycerin incubated anaërobically. Luxuriant growth as a heavy granular sediment on the bottom of the tubes, often extending up the sides for some distance. No pellicle. Broth usually clear but may rarely be clouded. The sediment often has a pinkish or brownish tint. Under aërobic conditions growth is slow and occurs only with large inoculums. Moderate growth in plain hormone broth.

*Milk.*—Coagulation with a soft curd and an expression of whey, under both aërobic and anaërobic conditions. The coagulation may be incomplete and require heating.

*Extract Gelatin*, pH 6.6. No growth.

*Potato.*—Moderate expansion, usually only under anaërobic conditions, deep pinkish in color. Invisible growth according to Gilchrist.

*Loeffler's Blood-serum and Dorset's Egg Medium.*—Good growth under anaërobic conditions. Pigmentation deeper than in cultures in dextrose or glycerin agar. Loeffler's serum shows a blackish-blue tint.

*Ground Cooked Meat.*—Visible growth only in the top layers of meat beneath the supernatant fluid.

**Virulence.**—Gilchrist's cultures were virulent to white mice and guinea-pigs, producing purulent foci.

1. Unna: The Histopathology of the Diseases of the Skin, 1896.
2. Sabouraud: Les maladies seborrheiques. Paris, 1902.
3. Gilchrist: Johns Hopkins Hosp. Rep., 1900, ix, 409.
———: J. Cutan. Dis., 1903, xxi, 107.

4. Hallé and Civatte: Ann. de dermat. et syph., 1907, viii, 185.
5. Ketron and Burky: Archiv. of Dermat. and Syph., 1925, xi, 354.
6. Fisk and Burky: J. Infect. Dis., 1922, xxx, 128.

### CORYNEBACTERIUM HOAGII (Morse)

Noted first as "Organism X" by Hoag[1] from the eye, throat, lungs, empyema cavities, stomach, liver, etc. Most frequently found in the lungs. Named *Bacillus Hoagii* by Morse[2] and put in Group A.

**Morphology.**—Small straight or curved bacilli, rarely showing suggestions of rudimentary branchings. Smaller than the diphtheria bacillus or Hofmann's bacillus. Often coccoid or conical. Involution forms common, pear-shaped, clubbed, large spheres. Arranged in palisade formation, open V or as jackstraws. Granules common, unipolar, bipolar, or central. Barred and streptococcus-like organisms not rare.

**Staining Reactions.**—Young forms stain well. Gram-positive, but some organisms are pale by this stain. Involution forms retain Gram's stain deeply. Granules brought out by Neisser's stain, smaller than in the diphtheria bacillus.

Non-motile. No capsules.

**Cultivated** easily on the usual media.

*Blood-serum.*—Growth more moist and creamy than that of the diphtheria bacillus and development more rapid. Many strains show a pale pink-salmon color. When old the growth is a very faint buff in color. Rarely yellow.

*Agar.*—Growth more rapid and moist than with diphtheria, usually gray, sometimes very faint salmon color.

*Gelatin.*—Growth slow. Very fine disseminated spherical colonies appear in the depths, surface growth dry, yellowish, and elevated.

*Potato.*—Faint salmon-pink or salmon-buff expansion.

Good growth in plain and in sugar broth. Marked turbidity with or without a pellicle, but with usually a marginal ring growth.

Highly acid in glusose, saccharose, and maltose (Hoag), in glucose and saccharose, but not maltose or glycerin according to Morse.

Usually non-pathogenic to guinea-pigs, rabbits, and white rats. Hoag observed purulent inflammation and death occasionally in guinea-pigs. The organisms were cultivated from the lesions and from the lymph-glands.

1. Hoag: Boston M. and S. J., 1907, clvii, 10.
2. Morse: J. Infect. Dis., 1912, xi, 253.

### CORYNEBACTERIUM FLAVIDUM (Morse)

Described by Morse[1] as *Bacillus flavidus* and put in her Group B of the diphtheroids.

*Morphology.*—Irregular bacilli, thick forms with clear-cut bars predominating. Size, 0.75 to 1.0 $\mu$ by 3 to 5 $\mu$ (Bergey[2]). Gram-positive. Neisser's granules very large and irregular.

*Cultivated* easily. Growth on serum heavy, varies in color from white to yellow, often noticeably dry and granular. No liquefaction of gelatin. No growth on potato.

Acid formed always from glucose, usually maltose and glycerin, not from saccharose.

1. Morse: J. Infect. Dis., 1912, xi, 253.
2. Bergey: Manual of determinative bacteriology, 1923, p. 384.

### CORYNEBACTERIUM RUEDIGERI (Mellon)

Isolated by Ruediger[1] from cases diagnosed as diphtheria, gangrenous tonsillitis and pharyngitis complicating scarlatina, black scarlet fever, and black diphtheria. Treatment with antidiphtheria serum had a bad influence on the patients, aggravating the symptoms. The organisms resembled the true diphtheria bacillus and were found in abundance in the throats.

Culturally they differed by the uniform turbidity produced in broth, soft, moist, and whitish growth on agar, hardy, light brown, growth on potato. Litmus milk was turned white in five to six days. Sugars not fermented to acid (Hoag[2]). Pathogenic to guinea-pigs from bacteremia when injected intraperitoneally in doses of 4 to 5 c.c. With one strain the viru-

lence was increased somewhat by animal passage, the fatal dose for a 250-gram guinea-pig being reduced to 1.5 c.c. of a twenty-four-hour broth culture. Animals were not protected by treatment with antidiphtheria serum, but were protected by treatment with serum made from one of the strains in rabbits. Subsequently obtained by Hamilton[3] from cases diagnosed as diphtheria in which diphtheria antitoxin had no curative effect, from the normal throat, from pharyngitis in measles, from the ear in scarlet fever, and from mild cases of supposed diphtheria.

Named *Bacillus Ruedigeri* by Mellon.[4]

1. Ruediger: Tr. Chicago Path. Soc., 1903, vi, 45.
2. Hoag: Boston M. and S. J., 1907, clvii, 10.
3. Hamilton: J. Infect. Dis., 1904, i, 690.
4. Mellon: J. Bact., 1917, ii, 269.

### CORYNEBACTERIUM DIPHTHEROIDES LIQUEFACIENS (Graham-Smith)

Found by Graham-Smith[1] in considerable numbers in the mouth of a patient suspected to be suffering from diphtheria.

On serum minute rounded colonies are formed in twenty-four hours, but in forty-eight hours they are medium-sized, round, slightly yellowish, dome-shaped, and opaque. After ten days' growth the colonies have sunk into slight pits, and the medium become partially liquefied after being kept twenty to thirty days at room temperature. The organisms are very long, markedly curved, and lie in groups more or less parallel to one another. There is very little clubbing, and but slight signs of segmentation, but all show well-marked terminal and other polar bodies. Some specimens remain as unstained shadows. They are motile, but the movements are slow. They retain Gram's stain. These organisms bear a fairly close resemblance to the diphtheria bacillus. On agar stroke cultures in twenty-four hours a thick, moist, smooth, slightly yellow abundant growth is formed. The appearance of the organisms is the same as on serum. On the surface of agar stab cultures an extensive moist smooth growth occurs, which occasionally in old cultures shows concentric markings. In the depth the colonies run together to form a continuous growth, the discrete colonies at the edges are rounded, but have blunt projections. On alkaline potato agar in forty-eight hours round, smooth, moist, dome-shaped, slightly yellowish colonies are formed, and the same is the case on acid potato sugar. On gelatin very minute, almost transparent colonies are formed. The medium becomes liquefied round them in ten days. In a few more days liquefaction is complete with a whitish-yellow mass lying in clear fluid. On gelatin stabs in three days the small yellowish surface growth is lying in a small cup-shaped area of liquefaction. In eleven days there is a deep funnel-shaped hollow with yellowish growth at the bottom and very minute colonies along the lower part of the needle track. On potato in three days a thin extensive white growth is formed, which in six days is very abundant and yellow. The organisms are of medium length, markedly curved, thin, and stain uniformly. Many are clubbed and the polar bodies are very minute. Broth in forty-eight hours is slightly cloudy, with a large deposit of finely granular matter. In forty-eight hours the reaction of glucose broth is neutral or faintly alkaline. In six to eight days litmus milk is decolorized and firmly clotted. No gas is produced, but much indol is formed and nitrates reduced. It is non-pathogenic.

The organism isolated by DeWitt[2] from tonsillitis is possibly the same species.

1. Graham-Smith: J. Hyg., 1904, iv, 258.
2. DeWitt: J. Infect. Dis., 1910, x, 36.

### CORYNEBACTERIUM SEGMENTOSUM (Cautley)

Isolated originally by Cautley[1] and obtained by Graham-Smith[2] from the nasal secretions of 3 persons suffering from coryza. Graham-Smith's description follows:

On serum grows more slowly than the diphtheria bacillus, though the colonies are very similar in appearance. Organisms in twenty-four hours resemble pseudodiphtheria bacilli with light median bands, but are longer. A considerable number of long forms resembling uniformly stained diphtheria bacilli are also found. They are non-motile, retain Gram's stain, and show small terminal polar bodies in most specimens. In subcultures many long segmented forms are seen, with two or four well stained segments, as well as shorter Hofmann-like forms. On agar in forty-eight hours, round, smooth, white, raised, dome-shaped colonies are formed. The organisms are long, well segmented, curved and clubbed, and but few short forms occur. Polar bodies are only found in a few. They resemble closely the diphtheria bacillus. In agar stab cultures in twenty-four hours a small, white, round, smooth surface

growth is formed, which is very extensive after forty-eight hours. There is well-marked growth in the depth. On alkaline potato agar in twenty-four hours very small, round, smooth, gray, dome-shaped colonies occur, which in forty-eight hours are large, smooth, white, and well raised above the surface. On acid potato agar a similar growth occurs. On gelatin at 22° C. small, round, white colonies are produced. On potato an almost invisible, thin, whitish growth is formed. Broth remains clear, and a stringy white deposit is found at the bottom of the tube. Glucose broth in forty-eight hours is very faintly acid. Non-pathogenic.

1. Cautley: Rep. Med. Off. Local Gov. Bd., 1894–95, p. 455.
2. Graham-Smith: J. Hyg., 1904, iv, 258.

### CORYNEBACTERIUM DIPHTHEROIDES CITREUS (Graham-Smith)

Isolated by Graham-Smith from the throats of five healthy children, two attending infected, and three, non-infected schools.

On serum in twenty-four hours the colonies closely resemble those of the diphtheria bacillus, but are rather larger and more opaque. After three days' growth they become slightly yellowish. The organisms are fairly long and stain darkly, in shape resembling Hofmann's bacilli, but with little trace of a median band. The majority are slightly curved and show small terminal polar bodies, though in some of the longer forms they are very large and distinct. Non-motile, and retain Gram's stain. In forty-eight hour subcultures a number of long forms with three or four well-marked segments and distinct polar bodies occur. On agar in twenty-four hours large, white, round, smooth, moist, dome-shaped colonies are formed. The organisms are the same in appearance on serum, but the polar bodies are better marked. In agar stab cultures a flat, thick, moist, white surface growth with indented edges is formed. The indentations are very evident after forty-eight hours' growth. Along the needle track the growth is well-marked. On alkaline potato agar large, round, white, smooth, dome-shaped colonies are formed. On acid potato agar the growth is similar. No growth was obtained on gelatin. On potato in twenty-four hours a very extensive pale yellow moist growth occurs. The organisms are mostly short, but a number of markedly clubbed and segmented forms are found, up to 6 or 8 segments. All show large and distinct polar bodies. Broth after forty-eight hours is clear with white, rather stringy deposit. In glucose broth the deposit is copious and white in large flocculent masses, which tend to stick to the sides. The reaction is very acid. Non-pathogenic.

Graham-Smith: J. Hyg., 1904, iv, 258.

### CORYNEBACTERIUM DIPHTHEROIDES BREVIS (Graham-Smith)

Obtained by Graham-Smith from a large abscess cavity opening into the mouth.

The colonies on serum in twenty-four hours are small, smooth, and white, but not so well raised as those of the diphtheria bacillus. Subsequently they develop somewhat filmy edges. The organisms resemble Hofmann's bacilli in shape, but are slightly curved and clubbed and show segmentation. The segments stain darkly, but the intervening bands except the middle one are not very definitely marked. They are non-motile, retain Gram's stain, and show polar bodies by Neisser's method. In subsequent subcultures segmentation is a marked feature. On agar stroke cultures an abundant, white, soft, slimy growth is produced in twenty-four hours. The organisms are long, well curved, clubbed and segmented, and show good polar bodies resembling those of the diphtheria bacillus. Agar stab cultures show an abundant white slimy surface growth which frequently becomes coarsely granular after further growth. A confluent abundant growth occurs along the needle track with numerous projecting colonies. On alkaline potato agar in twenty-four hours large, round, smooth, gray, dome-shaped colonies are formed, which in forty-eight hours are white and considerably larger. The organisms vary from short oval to long well-segmented forms. On acid potato agar the colonies are similar. On gelatin in three days medium-sized, round, smooth, dry-looking colonies with raised centers develop. On gelatin stab cultures a large, white, dry, granular surface growth is formed, and small round colonies develop in the needle track. On potato in twenty-four hours an extensive growth, thick, soft, and cream-colored which gradually becomes granular and slightly yellowish. The organisms vary in appearance, some are short, but many long and well-segmented with good polar bodies occur. In forty-eight hours broth becomes slightly cloudy and there is a fine whitish granular deposit. The reaction of glucose broth is extremely acid. Milk becomes partially coagulated in a few days and indol is produced. It is non-pathogenic.

Graham-Smith: J. Hyg., 1904, iv, 258.

### CORYNEBACTERIUM MACULATUM (Graham-Smith)

Found once by Graham-Smith in a culture from the throat of a possible contact.

On serum in twenty-four hours the colonies are opaque white, but otherwise resemble those of the diphtheria bacillus, although some short forms occur. The sides in some have slight bulgings at intervals. Numerous darkly-stained segments cross the bacillus transversely in most of the organisms, but in a few there are oval segments. In some bacilli, especially in the later subcultures, long unstained spaces are seen. The organisms are non-motile, and retain Gram's stain very deeply. Each bacillus shows numerous polar bodies by Neisser's method; some of these are large and round, others are elongated transversely across the bacillus, whilst others are very minute. These minute polar bodies are often very densely aggregated. The name indicates the remarkable spotted appearance seen when the organisms are stained by Neisser's method.

In the first culture the organism lay in tangled masses of 10 to 50 individuals. On agar in twenty-four hours minute, round, transparent colonies are formed which subsequently grow very slowly. The organisms are about one-third the length of those found on serum cultures and of various shapes, from oval to bloated pear-shaped bodies. The polar bodies are few. On the surface of agar stab cultures an almost transparent film is formed, but in the depth medium-sized yellowish colonies grow. On alkaline potato agar rounded, small, rather flat, slightly granular colonies with irregular edges are produced. The growth on acid potato agar is similar. On gelatin after ten days' growth the colonies are so minute as to be scarcely visible with a lens. Exceedingly minute colonies also form in the depth of gelatin stab cultures. On potato there is no visible growth. Broth remains clear, but a few very large, discrete, yellow granules (0.5 cm. in diameter) are seen after forty-eight hours. The reaction of glucose broth becomes very faintly acid. Milk remains unchanged and no indol is formed. The organisms are non-pathogenic when injected either subcutaneously or intraperitoneally.

Graham-Smith: J. Hyg., 1904, iv, 258.

### CORYNEBACTERIUM AURIS (Graham-Smith)

Isolated by Graham-Smith from the ear discharges of three scarlet fever patients.

On serum the colonies closely resemble those of the diphtheria bacillus, but grow more slowly. After forty-eight hours' growth the colonies are medium-sized. After thirty hours' growth the organisms are of various sizes, the majority of over medium length with darkly staining ends. They stain well, showing well-marked segments with intervening light bands. In the longer forms several segments occur. Nearly all, even the shorter forms, are well curved, and have a tendency to be clubbed. A few pear-shaped forms were met with. They are non-motile, retain the stain by Gram's method, and show several well-marked polar bodies by Neisser's stain. In subcultures the resemblance to diphtheria bacilli is still more marked. Nearly all the specimens are long, well segmented, and show several good polar bodies. The general arrangement in the field is similar to that of the diphtheria bacillus. On agar slopes in twenty-four hours the colonies are small, round, gray, and dome-shaped. The organisms are short, curved and clubbed with well-marked polar bodies. Agar stab cultures in twenty-four hours show a smooth, moist, white surface growth, and a good growth of discrete round colonies along the line of puncture. On alkaline potato agar in twenty-four hours, smooth, gray, round, dome-shaped colonies appear, which later become large and white. The organisms are long, clubbed, and segmented. On acid potato agar the colonies are smaller, but similar. On gelatin small, almost transparent, round colonies are formed in forty-eight hours, which in three to four days become large and white, with a smooth surface slightly elevated in the center. The growth is sticky and tenacious. The organisms are the same as on serum. On gelatin stabs in three days there is an irregular, granular, white surface growth faintly marked by concentric rings, and good growth in the depth. On potato in twenty-four hours there is a slight brownish-yellow growth which in forty-eight hours becomes extensive, soft, yellow, and glistening. The organisms are short and oval, and stain well with large terminal polar bodies. Broth in forty-eight hours is slightly cloudy with a white stringy deposit. In glucose broth a copious, finely granular deposit occurs, and the reaction is very markedly acid. Milk remains unchanged. Indol is formed. The organism is non-pathogenic.

Graham-Smith: Jour. Hyg., 1904, iv, 258.

### CORYNEBACTERIUM CERUMINIS (Graham-Smith)

Obtained by Graham-Smith from 13 specimens from normal ears, and from the ears of scarlet fever patients. Eleven of these were fully investigated. The organisms appeared to be more plentiful when ceruminous secretion was present on the swabs. From this circumstance the name has been given.

On serum in twenty-four hours the growth is scarcely visible, but in forty-eight hours small round colonies, indistinguishable from those of the diphtheria bacillus, are formed. After seventy-two hours the colonies are medium-sized to large. Two forms, slightly different in morphology were noticed. (*a*) In thirty hours long thin and curved, uniformly stained, with small terminal polar bodies. In seventy-two hours the bacilli are longer, more curved, well-segmented, and with well-marked polar bodies. (*b*) Medium length, slightly curved, uniformly stained, but markedly clubbed, with large polar bodies in a few specimens. After seventy-two hours the appearances of the two forms are similar. These organisms are non-motile, and retain the stain by Gram's method.

On agar after twenty-four hours the colonies are small, round, gray, and dome-shaped, but later become large and white. The organisms are of medium length, curved, often clubbed, fairly well segmented, and show good polar bodies. On agar stabs a small, white, moist surface growth is present in twenty-four hours, which after forty-eight hours' growth is often lightly marked with concentric rings. A confluent growth takes place in the line of puncture.

On alkaline potato agar in twenty-four hours medium-sized, opaque, white, smooth, dome-shaped colonies appear. On acid potato agar the growth is similar, but more copious. No growth was obtained on gelatin. On potato in twenty-four hours a whitish to yellowish-white growth occurs, later becoming abundant and yellow. After about ten days the growth has a dry granular appearance. The organisms are mostly short, slightly curved, and clubbed. Broth remains clear and a small, stringy, white deposit is formed. In glucose broth the deposit is granular and the reaction neutral or alkaline. Milk remains unchanged and no indol is formed. They are non-pathogenic.

Graham-Smith: J. Hyg., 1904, iv, 258.

### CORYNEBACTERIUM DIPHTHEROIDES GALLINARUM (Graham-Smith)

Isolated by Graham-Smith from the throat of a fowl. There was a hard tumor on the side of the left mandible, but the bird was otherwise normal.

On serum the colonies after twenty-four hours' growth resemble those of the diphtheria bacillus. Later the margins become crenated. In the first cultures the organisms were long, curved, and clubbed, with 3 or 4 well-marked polar bodies. Slight swellings were present round the polar bodies. The rest of the protoplasm stained lightly, but slight signs of segmentation were present. They resembled closely the diphtheria bacillus. In subcultures well-marked segments are seen. These organisms are non-motile, and retain the stain deeply by Gram's method. On agar, small, filmy, transparent, gray colonies are formed. Polar bodies are present. On agar stab cultures an almost transparent film is formed on the surface, and minute round colonies along the needle track. On gelatin after five days' growth, minute, round, almost transparent colonies appear. On gelatin stab cultures there is very little surface growth, and a very scanty growth of minute colonies along the needle track. On alkaline potato-agar in twenty-four to forty-eight hours very small, smooth, or slightly granular, rounded, almost transparent colonies are formed. On acid potato agar the growth is similar. No visible growth occurs on potato. Broth remains clear and there is a slight granular deposit. Glucose broth shows a neutral or slightly alkaline reaction. Indol is produced. Non-pathogenic to guinea-pigs.

Graham-Smith: J. Hyg., 1904, iv, 258.

### CORYNEBACTERIUM CUCULI (Graham-Smith)

Cultivated by Graham-Smith from the throat of a cuckoo (Cuculus canorus), and from that of a parrakeet.

On serum after twenty-four hours' growth the colonies closely resemble those of the diphtheria bacillus. The organisms varied in appearance, some were of medium length, curved, clubbed, and stained throughout, others resembled elongated Hofmann's bacilli with a central light band. Shadowy unstained forms were common. In subcultures the resem-

blance to the uniformly stained diphtheria bacillus is more evident. They are non-motile, retain Gram's stain, and show small terminal polar bodies by Neisser's method. On agar, large, smooth, white, dome-shaped colonies are formed in twenty-four hours. The organisms are long, much curved, clubbed, and well segmented, but show no polar bodies. On agar stab cultures an extensive, white, heaped-up surface growth occurs and numerous colonies along the line of puncture. On alkaline potato agar round, smooth, dome-shaped, opaque, white colonies appear. The colonies on acid potato agar after twenty-four hours' growth are similar, but after four days' growth a thin, filmy, broad expansion appears round the central mass. On gelatin no growth was obtained, and on potato no visible growth occurred. Broth becomes slightly cloudy, and there is a finely granular deposit. After forty-eight hours the reaction of glucose broth is neutral. Non-pathogenic.

Graham-Smith: Jour. Hyg., 1904, iv, 258.

### Corynebacterium Hodgkini Bunting and Yates

Pseudodiphtheria bacilli or diphtheroids were first observed in Hodgkin's disease by Fraenkel and Much,[1] who treated the nodes with antiformin and

Fig. 21.—*Pseudodiphtheria bacillus* from Hodgkin's disease, Bunting strain, eight-day culture on blood-serum, stained with methylene-blue.

found granular, Gram-positive, non-acid-fast bacilli in the sediment in 12 out of 13 cases examined. Similar organisms were first cultivated by de Negri and Mieremet[2] in 2 cases on Bordet's medium (blood-glycerin-potato agar). They agreed in morphology with the forms described by Fraenkel and Much and were named *Corynebacterium granulomatis maligni* by Negri and Mieremet. They grew luxuriantly at body temperatures under both aërobic and anaërobic conditions. Diphtheroids were first cultivated in America from Hodgkin's disease by Bunting and Yates[3] and named by them *Corynebacterium Hodgkini*. They were subsequently obtained from Hodgkin's disease by Billings and Rosenow,[4] and by Rhea and Falconer.[5] The strain regarded as most characteristic by Bunting and Yates grew readily at 37° C. on Dorset's egg medium, glycerin-phosphate agar, Loeffler's blood-

serum, and on ordinary agar. On glycerin-phosphate agar the growth was almost as luxuriant under strict anaërobic conditions as under aërobic.

Marked moisture of the media was necessary to secure luxuriant growth. On relatively dry media growth was slow and the organisms appeared as long bacilli, granular, banded, with many club-shaped involution forms. Branching forms were also noted. On egg medium the organisms tended to cohere and in stained smears many small groups of organisms radially arranged were found, with clubbed peripheral elements, somewhat suggestive of a minute actinomyces colony. On moist serum tubes with luxuriant growth the organisms were short and plump with polar staining. Many of these forms were coccoid. In old cultures coccoid forms predominated and large spherical involution forms were present. A colony or a streak showing in twenty-four hours entirely the bacillary form will twenty-four or forty-eight hours later show an apparent outnumbering of the bacilli by the coccoid elements. The organism was Gram-positive, the shorter plump forms holding the dye less strongly than the longer bacillary form. Non-acid-fast. No spores.

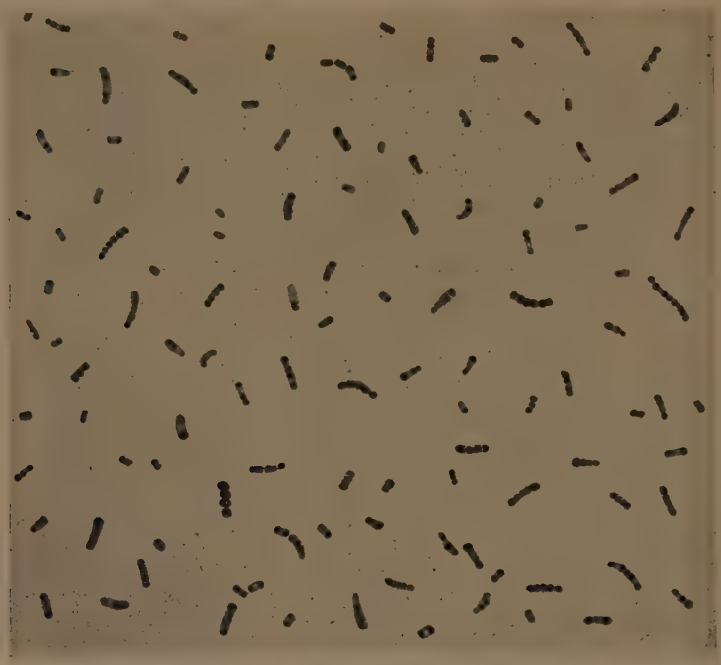

Fig. 22.—*Pseudodiphtheria bacillus* from Hodgkin's disease, Bunting strain, eight-day culture on plain agar, stained with methylene-blue.

The growth of the organism was at first glistening and grayish, later becoming more opaque and white. In some early cultures a slight greenish-yellow tint was produced. Old cultures on glycerin-phosphate agar became brownish and the media itself was darkened.

No liquefaction of gelatin.

No early change in reaction in litmus milk.

Broth not clouded, flecks appearing along the sides of the tube, and a slimy deposit gradually accumulating at the bottom.

Plate cultures showed rounded colonies with quite regular edges, a fine stippling of the growth and a central dark spot. They were glistening, grayish in color at the end of twenty-four hours, gradually becoming opaque white.

**Pathogenic Action.**—With pure cultures Bunting and Yates[6] produced in monkeys a chronic progressive lymphadenitis closely resembling Hodgkin's disease in its histological features and subsequently a general glandular enlargement leading to the death of the animal.

1. Fraenkel and Much: Ztschr. f. Hyg., 1910, lxvii, 159.
2. de Negri and Mieremet: Centralbl. f. Bakteriol. [etc.], 1913, lxviii, 292.
3. Bunting and Yates: Arch. Int. Med., 1913, xii, 237.

4. Billings and Rosenow: J. Am. M. Ass., 1913, lxi, 2122.
5. Rhea and Falconer: Arch. Int. Med., 1915, xv, 438.
6. Bunting and Yates: J. Am. M. Ass., 1913, lxi, 1803.
7. Bunting and Yates: J. Am. M. Ass., 1914, lxii, 516.

### CORYNEBACTERIUM PARALYTICANS (Robertson, M'Rae, and Jeffrey)

Described originally by Robertson, M'Rae, and Jeffrey[1] from the respiratory and alimentary tract in autopsies on cases of general paralysis. Put by Morse[2] in Group B, but as fermenting saccharose.

**Morphology.**—Large barred bacillus, often with tapering ends and with numerous well-formed granules. Thicker and less curved than the diphtheria bacillus. Arrangement usually parallel. At the end of four days' growth the bacilli were fused into a poorly staining mass and no Klebs-Loeffler involution forms had developed.

Easily cultivated. Abundant growth of isolated, moist, yellow-white colonies on blood-serum, the largest 1 mm. in diameter. Growth more translucent than that of the diphtheria bacillus.

Produces much acid from dextrose, saccharose, and maltose, none from glycerin or dextrin (Morse).

Produces morbid phenomena in rats by feeding and lesions of the nervous system like those seen in general paralysis (Robertson[3]).

1. Robertson, M'Rae, and Jeffrey: Rev. Neurol. and Psychiat., 1903, i, 225; 305.
2. Morse: J. Infect. Dis., 1912, xi, 253.
3. Robertson: Brit. M. J, 1903, ii, 1065. *Ibid.*, 1905, ii, 1427.

### CORYNEBACTERIUM PLUMOSUM Fox

Isolated by Fox from the blood in a cryptogenic infection.

**Morphology.**—Moderately long slender bacilli, 1.5 to 3.3 by 0.67 to 1.3 $\mu$ in dimensions, with thin or thin rounded ends. The protoplasm is granular and stains irregularly. Not definitely beaded. These rods seem to grow out into thin, irregularly faintly staining straight or wavy threads which may or may not have clubbed ends. These clubbed forms are more numerous in agar cultures more than seventy-two hours old, but are abundant in gelatin kept at 37° C. for forty-eight hours. The threads average 7 $\mu$ in length and 1.3 $\mu$ in width, the largest observed being 35 $\mu$. The clubbed ends stain more deeply than the threads. They are 1.3 to 1.6 $\mu$ wide and of varying length. Both the rods and threads contain metachromatic granules, about one-third from the ends in the rods. Cuneate forms also appear, 2 by .67 $\mu$ in size, staining very uniformly. No branching. Non-motile. No flagella.

**Staining Reactions.**—The variations in morphology noted above appear with Loeffler's methylene-blue. The organisms stain uniformly with dilute carbolfuchsin or simple basic fuchsin. Gram-negative. Not acid-fast. Carbol thionin and dilute thionin give results similar to, but not precisely like, Loeffler's stain. In young cultures the rods are arranged radially about a center. In forty-eight hours these radiating growths have largely disappeared and one finds double rows of parallel rods which seem to branch from an axis. This appearance is not unlike a feather.

**Cultural Characters.**—*Agar Slant.*—Narrow, raised, translucent, irregular, uneven, pale creamy-white, glistening, filiform streak in twenty-four hours. Growth more luxuriant in forty-eight hours, but never as good as that of *Bacillus coli.*

*Blood-serum.*—Barely visible, slightly raised streak apparently made up of isolated colonies.

*Potato.*—Non-visible growth.

*Litmus Milk.*—Very faint acidity if any change occurs, no sediment, no coagulation.

*Broth.*—Faint turbidity beginning at the top and rapidly going downward, at first made up of clouds, then fine granules, and at fifteen days showing a heavy granular sediment (chiefly débris and broken filaments). No indol.

*Gelatin.*—37° C. At a fine turbidity apparently made up of minute granules chiefly at surface and spreading slowly down. No liquefaction in three weeks.

*Sugars.*—No gas production. Acid from dextrose, saccharose, maltose, and mannite. None from lactose and glycerin.

Nitrates not reduced to nitrites. No odor.

Optimum temperature 37° C. Perceptible growth at room temperature after four days. Killed at 50° C. in twenty minutes and at 60° C. in one minute.

Non-pathogenic to guinea-pigs, rabbits, and rats.

Fox: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1913, lxx, Orig., 143.

### CORYNEBACTERIUM ENZYMICUM (Mellon)

Obtained from the pleural cavity in an obscure case of lung infection by Mellon.[1] Agglutinated by the patient's serum in a dilution of 1 to 80 as against 1 to 15 for normal sera. The organisms also gave the complement-fixative reaction with patient's serum in 0.1 c.c. quantities as against 0.35 c.c. for normal sera. Probably the same as the organism described by Walker and Adkinson.[2]

**Morphology.**—Irregular diphtheroid bacillus of the barred type from blood agar slants, measuring 0.7 to 3 $\mu$ and containing 2 to 5 bars.

**Staining Reactions.**—Stains well with methylene-blue and is strongly Gram-positive. Cultivated in 1 per cent. glucose veal broth with a few drops of sterile human or rabbit serum the organism undergoes a striking change in morphology from a barred bacillus to a coccoid or diplococcus form with occasional short chains. The coccus or diplococcoid form can be converted to the barred bacillary type by cultivating aërobically on partly desiccated blood agar or on media containing varying quantities of acid, or an excess of alkali. The bacillary form is acid-fast, resisting decolorization in a weak acid-alcohol solution (1 per cent. HCl in 20 per cent. ethyl alcohol), while the diplococcus form will not hold the carbolfuchsin under these conditions. The diplococcus form is Gram-positive and the bacillary form Gram-negative.

Non-motile. No spores.

Capsules present in the bodies of animals.

Grows as a facultative anaërobe. Has intense viability, resisting desiccation and the addition of antispetic substances to the media. Destroyed at 58° to 60° C. in three minutes. Not killed by long exposure to sunlight. Grows at room temperature and at 37° C.

*Blood Agar Slants.*—Very fine transparent colonies in twenty-four to forty-eight hours, later becoming translucent or gray. If the medium be kept moist the colonies often become opaque and increase greatly in size. The fine transparent colonies show bacillary forms, the opaque colonies, cocci and coccoids.

*Glucose Broth.*—Sparse granular growth settling to the bottom of the tube and clinging to the walls. It may become flocculent or nebulous if serum be present in the medium.

*Litmus Milk.*—Acidity in twenty-four hours, firm coagulation in forty-eight to seventy-two hours.

No growth on potato.

Acid in glycerin, glucose, lactose, maltose, saccharose, dextrin, inulin, and salicin.

Growth good in gelatin without liquefaction.

In ascites broth the growth is good with a marked tendency to form branching and fusing involution forms.

No gas formed. No indol. Nitrites formed in traces.

All media give better results with the addition of 1 per cent. glucose. The acid formed in glucose broth is not sufficient to kill the organism after seven to ten days at 37° C. After the available sugar is used up the proteins of the media are attacked with a resulting alkaline reaction.

**Pathogenic Action.**—Virulent to rabbits by intravenous inoculation, the animals showing various lesions, acute catarrhal cholecystitis, acute suppurative arthritis, degenerative myositis, and parenchymatous nephritis. The organisms could be recovered from the blood and the various organs. Filtrates of broth cultures were also toxic, producing a loss in weight and cachexia. This toxic substance was destroyed at 85° C. in half an hour.

1. Mellon: Med. Rec., 1916, lxxxix, 240.
   ———: Am. J. M. Sc., 1915, cl, 245.
   ———: J. Bact., 1917, ii, 81.
2. Walker and Adkinson: J. Med. Research, 1917, xxxv (n. s. xxx), 295.

### CORYNEBACTERIUM PYOGENES (Glage)

Organisms producing suppuration in cattle were described by Lucet[1] and Künneman[2] as *Bacillus pyogenes bovis*, in swine by Grips[3] as *Bacillus pyogenes suis*. The organisms found by Grips and by Künneman were regarded as identical by Glage[4] who proposed the name *Bacillus pyogenes* or *Bacterium pyogenes*. First described in this country by Ward[5] who isolated it on beef serum agar plates. Subsequently investigated by Brown and Orcutt[6] who studied 12 different strains and placed the organism in the group *Corynebacterium*.

**Morphology.**—Very minute, non-motile rods varying in size from 0.3 to 2 $\mu$ in length and about 0.2 $\mu$ broad. Chains are not infrequent and tapering forms may occur. Spores not formed. Brown and Orcutt find that the organisms occur as:

1. Small, short, homogeneously stained bacilli arranged singly.
2. Fusiform organisms with Gram-positive central granules, often slightly curved.
3. Diphtheroid forms, bacilli of irregular length and contour, some clubbed and containing deeply stained bands or granules.
4. Streptococcoid form. Diplococci and streptococci in small clumps and short, crooked chains.
5. Filamentous and branching forms of irregular diameter, also definitely budded forms. They are likely to be mistaken for streptococci.

**Staining Reactions.**—Stained readily by ordinary basic anilin dyes. Stains well, but irregularly with dilute carbolfuchsin. With alkaline methylene-blue, banded forms are common, and long forms which display darkly stained areas at the ends. Stains uniformly brown with Neisser's diphtheria stain. Gram-positive, but not so strongly so as staphylococci. Dead organisms Gram-negative.

**Cultural Characters.**—*Bacterium pyogenes*, according to Berger,[7] grows at temperatures from 24° to 40° C. and best at 37° C. Growth is possible only with certain media and is best in media containing serum and in milk.

*Serum Agar.*—Growth appears in thirty-six to forty-eight hours at 37° C. as minute points, maximum growth being reached in several days. Surface colonies are scanty and rarely exceed 3 mm. in diameter. They are coarsely granular under low power in the center, more uniform toward the edges. Edges entire. Deep colonies irregular in shape, trefoil-like, heart-shaped, rarely lenticular. Under low power the borders are sharp, the surface slightly granular. Old colonies are surrounded by a halo-like cloudiness.

*Serum Bouillon.*—Cloudiness of fine flocculent grayish flakes which eventually deposit as a sediment in the bottom and along the sides of the tube. The supernatant fluid is more or less clouded, according to the amount of serum present. The sediment consists of masses of agglutinated bacteria. When shaken these produce an irregular clouding of the serum.

Fair growth in plain standard veal infusion broth made with Fairchild's peptone. Turbidity in twenty-four hours (Brown and Orcutt).

*Beef Serum Slants.*—Pit-like depressions or trough-like excavations after several days from liquefaction of the serum. The fluid serum is at first cloudy, then clears with a whitish sediment. The mass of serum is gradually and eventually almost completely liquefied.

*Milk.*—Acid production at 37° C. in forty-eight hours with complete coagulation in three days. With indicators such as rosolic acid and china-blue or bromcresol purple, the milk is seen to remain acid for at least three weeks till the coagulum is completely digested. Coagulation occurs at a titratable acidity far below that required of hydrochloric or acetic acid to coagulate milk. Soluble casein is also coagulated in neutral or slightly alkaline sugar-free medium. Coagulation of milk by *Bacterium pyogenes* is thus due to an enzyme. The digestion stops short of ammonia production. In milk three weeks old no increase of ammonia is shown by Folin's method.

Growth also on Dorset's egg medium, the colonies producing little pits like those seen on coagulated serum. Grows well on a medium consisting of three parts of white of egg and one part of standard veal infusion bouillon, slanted and coagulated in the inspissator (Brown and Orcutt). Depressions similar to those on coagulated serum.

According to Brown and Orcutt, *Bacterium pyogenes* produces hemolysis on standard veal infusion agar plus 5 to 10 per cent. defibrinated horse blood. In twenty to twenty-four hours very minute colonies appear with very small zones of hemolysis about them. In forty-eight hours the deep colonies are quite small biconvex disks about 0.3 mm. in diameter, easily seen microscopically. The hemolyzed zones are clear, well-defined, colorless and of the Beta type of Smith and Brown, 1.5 to 2 mm. in diameter. Hemolysis of blood in salt solution occurs also, just like that seen with hemolytic streptococci of human origin.

**Fermentation Reactions.**—Fermented broth + 1 per cent. sterile horse serum + 1 per cent. sugars. Acid from dextrose, saccharose, lactose and xylose, but not from raffinose, inulin, mannite, and salicin (Brown and Orcutt). Holth has reported a fermentation of galactose, fructose, and maltose, but not of xylose. Brown and Orcutt used meat infusion while Holth used meat extract. Owing to agglutination of *Bacterium pyogenes* by horse serum in a dilution of 1 to 100 the growth is chiefly in form of a sediment in the neck of tube. Acidity higher in bulb than arm unless tubes be laid in horizontal position when acid is formed in both arm and bulb. Acidity and agglutination are not the factor. Plain broth containing carbohydrates also gives clouding in arm but no acid.

*Not Hemoglobinophilic.*—Hemoglobin least essential for growth. Serum probably more valuable.

Thermal death-point between 55° and 59° C. (Berger).

**Pathogenic Action.**—Slightly virulent to mice and guinea-pigs. Subcutaneous injection produces local abscesses in rabbits, and intra-abdominal inoculation an acute and chronic peritonitis. The animal may die acutely in six to fourteen days or develop chronic intoxica-

tion which results fatally after some weeks or months. Intravenous inoculation in rabbits may give rise to pyemia with metastatic foci in various organs including the peritoneum and joints. The lesions produced in animals are characterized by proliferation of connective tissue which produces tumor-like masses, in the middle of which necrotic changes occur leading to abscess formation. According to Brown and Orcutt, rabbits are most susceptible of all laboratory animals, guinea-pigs less so, mice least. Intravenous inoculation of rabbits is invariably fatal with lesions common in the bones. Paralysis frequent, from lesions in vertebræ exerting pressure against the ventral columns of the spinal cord. Muscle abscesses also frequent.

1. Lucet: Ann. de l'Inst. Pasteur, 1893, vii, 325.
2. Künneman: Arch. f. Wissensch. u. prakt. Tierh., 1903, xxix, 128.
3. Grips: Ztschr. f. Fleisch-u. Milchhyg., 1898, viii, 166.
4. Glage: Ztschr. f. Fleisch-u. Milchhyg., 1903, xiii, 166.
5. Ward: Jour. Bact., 1917, ii, 619.
6. Brown and Orcutt: J. Exper. M., 1920, xxxii, 219.
7. Berger: Ztschr. f. Infektionskr. . . . d. Haustiere, 1907, iii, 101. *Ibid*, 1908, iii, 356.

### DeWitt's motile gas-producing diphtheroid

Obtained by DeWitt from a case of phlegmonous tonsillitis complicated by otitis media and membranous vulvitis. The discharge from all sources had a very offensive odor and gave numerous cultures of the same organisms.

**Morphology.**—Bacilli somewhat shorter and of more regular form than the typical diphtheria bacillus, but very closely resembling it in morphology and staining characteristics. No thread formation, but the grouping is very similar to that of the Klebs-Loeffler organism. It stains readily with anilin dyes.

Some of the organisms show distinct polar bodies with Loeffler's methylene-blue and with the Neisser stain, but these bodies are mostly unipolar or the organisms stain uniformly throughout. With the Gram stain the stain washes out, but with considerable difficulty and in every preparation many of the organisms retain the stain. Motility active, especially in young broth cultures.

**Cultural Characters.**—The organism grows readily on most of the ordinary media, growing slowly at room temperature. Optimum temperature 37° C.

*Loeffler's serum* is the most favorable medium for its cultivation, giving a moist, soft, slimy, abundant, spreading growth, at first nearly colorless, later pale yellow.

The serum is completely liquefied in a few days.

*Potato.*—Abundant, moist, elevated, rapidly spreading, almost colorless growth.

*Agar Slant.*—Slightly yellowish, moist, spreading growth with a tendency to soften and liquefy the agar.

*Blood Agar.*—Fairly large, elevated, yellowish, moist colonies surrounded by a small area of hemolysis.

*Glucose Agar.*—Small amount of gas.

*Glucose Broth.*—Abundant gas formation.

No gas in lactose, maltose, or saccharose broth.

All sugar media give an alkaline reaction after seventy-two hours.

*Plain Broth.*—Rapid growth rendering the fluid cloudy at first. Later most of the growth settles to the bottom and sides as a granular grayish-white precipitate.

*Gelatin Stab.*—Rapid liquefaction, an inverted cone formed at first, in forty-eight hours the entire tube being liquefied, most of the growth settling to the bottom.

*Litmus Milk.*—Slow acid formation with coagulation at the end of seventy-two hours.

In all media a characteristic, peculiar, sweetish acetone-like odor is formed, which is like that noted in the patient.

Indol positive in forty-eight-hour peptone cultures. Slight phenol reaction. No reaction for nitrites.

**Pathogenic Properties.**—Subcutaneous inoculation of guinea-pigs with twenty-four- to forty-eight-hour cultures produces an indentation at the point of inoculation. This may soften and rupture. Intraperitoneal inoculation produces a low-grade peritonitis. Filtrates from forty-eight-hour broth cultures are pathogenic to guinea-pigs by intraperitoneal inoculation, the animals dying in thirty-six to forty-eight hours.

According to DeWitt this organism resembles Graham-Smith's *Bacillus diphtheroides liquefaciens.*

DeWitt: J. Infect. Dis., 1912, x, 36.

### CORYNEBACTERIUM LYMPHOPHILUM (Torrey)

Found in abnormal lymph-glands by Torrey, especially in Hodgkin's disease, and described as *Bacillus lymphophilus.*

**Morphology.**—Slender pleomorphic bacillus with rounded ends, some strains measuring 0.5 by 1.2 to 3.2 $\mu$, others 0.4 by 1 to 2 $\mu$. It generally occurs singly, at times in pairs, but never forms chains in any media. A smear from a primary culture deep in the medium frequently shows short, rather plump rods which vary little in length, measuring 0.75 to 1 by 1.5 to 2 $\mu$ and are generally slightly curved. In stab cultures in $\frac{1}{2}$ per cent. nutrient agar, which is a very favorable medium, the rods show more irregular morphology, vary in length and stain rather unevenly, the ends being frequently slightly swollen. They are collected in clumps. Deep-staining granules not observed, no barred or clubbed types. Growth in or on dry media may show a few rods with bifid ends. Non-motile.

**Staining Reactions.**—Strongly Gram-positive. Not acid-fast. Stains rather lightly with alkaline methylene-blue and with dilute carbolfuchsin.

**Cultural Characters.**—Optimum temperature 37° C. Slow growth at 30° C. and at 41° C. No growth at 20° to 25° C. Cultivated best by burying bits of tissue at various depths in a column of ascites glucose agar with a reaction of about +2 to phenolphthalein. This acid reaction is decidedly favorable and accelerates the growth of this bacillus. In a medium with a reaction of +.5 to +1 the rate of growth was very slow and growth was not observed in primary cultures until the eighth or tenth day, whereas with the more acid medium the same result was obtained in two to four days. The addition of ascitic fluid to the medium is not essential. From the tissue planted deep in the medium this bacillus grew out as a white papilla which increased in size until after a week or more a shelf-like structure had been formed, very similar in appearance to a fungus. This growth was tinged a faint pink and clouded the medium. It emitted a sour, musty odor. Colonies appeared at various depths of the medium up to within 1 cm. of the surface. The isolated colonies were angular, pyramidal in shape and not more than 2 mm. in diameter. Subcultures were most successful in deep stab inoculations $\frac{1}{2}$ per cent. nutrient agar with a reaction of about +2 to phenolphthalein. The zone of optimum growth in such media varied somewhat with different strains. With some the first growth was observed near the surface, in others half way down and with still others at the bottom of the tube. Most of the strains would finally grow up to the surface although rarely to any degree on the surface. Occasionally certain strains could be induced to develop on the surface of Loeffler's solidified serum in the form of a raised white growth, quite similar to that of *Staphylococcus albus.* Acid production with carbohydrates in fluid media was very high. In dextrose broth with an initial acidity of +2.3 to phenolphthalein the titre was raised by certain strains in ten days up to +10 or even +12. Almost as much acid was produced as by *Bacillus bulgaricus.* The organism is thus markedly aciduric and to a considerable extent acidophilic. It was found capable of multiplying in glucose broth containing 5 per cent. normal acetic acid. Glycerin was also fermented actively, the acid equaling or even exceeding that in dextrose broth. No action on lactose, mannite, inulin, or dextrin. Saccharose split by only 1 strain. No gas.

Growth in broth generally as a clouding of the lower third of the tube with a heavy sediment. With some strains there was an even clouding of the whole medium. Very slight growth in stab cultures in sugar-free media.

No action on litmus milk. No liquefaction of gelatin or serum.

No resistance to antiformin.

Non-pathogenic to guinea-pigs, rabbits, and monkeys. A monkey dying ten days after the inoculation, probably from other causes, yielded cultures of the bacillus from axillary and mesenteric lymph-nodes and from the spleen. An equal period of persistence occurred within the body of a guinea-pig.

Agglutinins produced easily in rabbits, the serums having an agglutinating power of two thousand to five thousand. The greater number of strains agglutinated to the same degree with each one of the several sera produced.

Regarded by the author as identical with Bloomfield's anaërobic diphtheroid.

Torrey: J. Med. Research, 1916, xxxiv (n. s. xxix), 65.

### BACILLUS D OF ADAMSON

An anaërobic diphtheroid isolated on one occasion by Adamson. It resembles *Bacillus fragilis* of Veillon and Zuber and *Bacillus gracilis putidus.*

*Morphology.*—On agar, short, coccus-like bacilli with round ends, isolated or in short chains of 3 to 6 which are frequently curved. No filaments. No spores. Gram-positive.

*Agar colonies* transparent, very small like streptococcus colonies. Older colonies larger, whitish, translucent, or semi-opaque.

*Glucose Agar.*—Colonies similar, but larger. Young colonies less transparent and rather sticky. Older colonies semi-opaque, white or pinkish. (Bacilli fatter and clubbed.)
*Glucose Agar Stab.*—Line growth, white, translucent. No gas.
*Dorset's Egg.*—Colonies minute, whitish or transparent, quite superficial. Do not erode surface. (Bacilli club-shaped.)
*Egg Broth.*—No change.
*Brain.*—No change. No gas. No odor.
*Meat.*—No change. No gas. No odor.
*Inspissated Serum.*—Minute surface colonies. No liquefaction.
*Broth.*—Slight turbidity. Clearing with deposit.
*Glucose Broth.*—Growth more active, greater turbidity. Sedimentation.
*Gelatin.*—No liquefaction.
*Milk.*—No change. Growth as gelatinous mass suspended near surface.
No fermentation of glucose, saccharose, maltose, lactose, or mannite.

Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.

## GENUS 3. FUSIFORMIS Hoelling

Obligate parasites. Anaërobic or microaërophilic. Cells frequently elongate and fusiform, staining somewhat unevenly. Filaments sometimes formed; non-branching. Non-motile. No spores. Growth in laboratory media feeble.

**Fusiform bacilli** were apparently first observed by Miller[1] in the human mouth and by Babes[2] in cases of angina resembling diphtheria. This angina was further studied by Plaut[3] in 1894 who found organisms in it larger than the diphtheria bacilli and pointed at the ends, which he referred to as "Miller's bacilli." The condition was described more completely by Vincent[4] in 1899 and since then it is known as Plaut-Vincent's angina or Vincent's angina. With it are associated fusiform organisms, usually called *Bacillus fusiformis*, together with other bacteria, staphylococci, streptococci, and spirochetes. According to Besson[5] the species described by Zeitz as *Bacillus hastilis* is really *Bacillus fusiformis.*

Mühlens and Hartmann[6] and Hoelling[7] have advanced some evidence that this organism is provided with true nuclei. On account of this and also because of the peculiar morphology, the fusiform bacilli from the mouth, from the intestines of termites, and from the blind gut of the mouse are combined in a new genus, *Fusiformis*, the mouth organisms being designated *Fusiformis dentium.*

Fusiform bacilli have been observed in a great variety of conditions in man, but we are by no means sure that they are the same organism. Thus in the mouth they have been seen in normal individuals by Vincent and Mühlens as well as in angina. They have been found in ulcerative stomatitis by Bernheim and Pospischill,[8] in noma by Róna[9] and Gins,[10] in abscesses of the upper jaw and lungs by Silberschmidt.[11] They are practically always associated with spirochetes and other mouth organisms. They are not very unusual in routine examinations of the throat. Abel[12] found them 6 to 8 times in several hundred cases and in Neisser's laboratory in Frankfurt they were present 3 times in 737 throat observations in one series and later 6 times in a period of eight months (Gins).

They have also been observed in the mouths of animals, as in the maul of a lion with slimy secretion.

In addition to the fusiform bacilli found in the mouth under normal and pathological conditions and in regions associated with the buccal cavity, they have been observed by Vincent in hospital gangrene, by Mühlens in erosive balanitis and on hard and soft chancres, in the diarrheal stools of children by Veillon and Zuber[13] and by Grigoroff[14] in the pus in appendicitis.

These are present practically always in the secretions of tropical ulcers, usually in combination with spirochetes.

The fusiform bacilli observed in such diverse locations have certain characteristics in common. They vary in length from 6 to 12 $\mu$ and are 0.6 to 0.8 $\mu$ in breadth, are thickened in the middle and pointed at the ends, but never club-shaped. They are usually straight, but may be slightly curved or S-shaped. Often arranged in pairs end to end. They may be stained by the basic anilin dyes, best by dilute carbolfuchsin or carbol-thionin. Gram-negative. When stained by methylene-blue or by Gram they show peculiar vacuoles in the body of the organism regarded by Vincent as areas of degeneration. Many cells show darkly staining areas in the middle of the body which may be brought out by Giemsa or polychrome methylene-blue, appearing red or reddish-blue with the cell plasm blue. They may also be stained by Heidenhain's iron hematoxylin, appearing homogeneous or with a regular structure. These structures are regarded as nuclei by Mühlens and Hartmann and by Hoelling. According to Hartmann the thick nuclear substance in the fusiform bacilli from the mouth may dissolve and split up into chromidia. The fusiform bacilli are non-motile and have no spores.

Pure cultures of fusiform bacilli were not originally obtained by Vincent. The organisms were first cultivated by Veillon and Zuber from the pus in appendicitis. They were subsequently cultivated from the buccal cavity by Ellermann,[15] Lewkowicz,[16] Weaver and Tunnicliff,[17] Tunnicliff,[18] Mühlens,[19] Vesprémi,[20] Peters,[21] Ozaki,[22] and by Krumwiede and Pratt.[23] Cultures were obtained only under anaërobic conditions, by Ellerman on horse-serum agar, by Weaver and Tunnicliff on serum agar, by Tunnicliff on ascites agar, by Mühlens (and Hartmann) on serum agar and glucose agar, by Peters on Dorset egg medium, by Ozaki on ascites sugar agar, and by Krumwiede and Pratt on a special alkaline medium containing agar and gelatin and horse serum or ascites fluid. According to Krumwiede and Pratt fusiform bacilli fall into two main groups, some fermenting saccharose and some failing to act on this carbohydrate.

The most complete description of the fusiform organisms from the mouth has been given by Ozaki, whose description we follow. For the present the name *Fusiformis dentium* should be limited to the mouth organisms since it is not entirely clear that the other fusiform bacilli are identical with it.

Type species *Fusiformis termitidis* Hoelling.
" *dentium* (Babes) Hoelling.
" *muris* Hoelling.

Fusiform bacilli in appendicitis.

1. Miller: Deutsche med. Wchnschr., 1888, xiv, 612.
2. Babes: Deutsche med. Wchnschr., 1893, xix, 1035.
3. Plaut: Deutsche med. Wchnschr., 1893, xx, 920.
4. Vincent: Ann. de l'Inst. Pasteur, 1899, xiii, 609.
5. Besson: Practical bacteriology, microbiology and serum therapy. Hutchens' Translation, Lond., 1913, p. 574.
6. Mühlens and Hartmann: Ztschr. f. Hyg., 1906, lv, 81.
7. Hoelling: Arch. f. Protistenk., 1910, xix, 239.
8. Bernheim and Pospischill: Jahrb. f. Kinderh., 1898, xlvi, 434.
9. Róna: Arch. f. Dermat. u. Syph., 1904, lxxi, 191.—*Ibid.*, 1905, lxxiv, 171.
10. Gins: *Bacillus fusiformis*. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, v, 1003.
11. Silberschmidt: Centralbl. f. Bakeriol. [etc.], 1. Abt., 1901, xxx, 159.
12. Abel: Centralbl. f. Bakteriol, [etc.], 1898, xxiv, 1.
13. Veillon and Zuber: Arch. de méd. expér. 1898, x, 517.
14. Grigoroff: Thèse de Genève, 1905, p. 66.
15. Ellermann: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1904, xxxvii, 729.—*Ibid.*, 1905, xxxviii, 383.

16. Lewkowicz: Centralbl. f. Bakteriol, [etc.], 1906, xli, 153.
17. Weaver and Tunnicliff: J. Infect. Dis., 1905, ii, 446.
18. Tunnicliff: J. Infect. Dis., 1906, iii, 148.—*Ibid.*, 1911, viii, 316.
19. Mühlens: Ztschr. f. Hyg., 1906, lv, 81.
———: Deutsche med. Wchnschr., 1905, xxxviii, 136.
20. Vesprémi: Centralbl. f. Bakteriol. [etc.], 1907, xliv, 332.—*Ibid.*, 1908, xl, 15.
21. Peters: J. Infect. Dis., 1911, viii, 455.
22. Ozaki: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, 76.
23. Krumwiede and Pratt: J. Infect. Dis., 1913, xii, 199.—*Ibid.*, 1913, xiii, 438.

### FUSIFORMIS DENTIUM Hoelling

Cultivated by Ozaki[1] from the mouth.

**Morphology.**—Organisms larger on solid media than in fluid, since they grow to long threads in early stages, while in broth the long threads appear later. Typical in forty-hour sugar agar stab cultures, .04 to 1 $\mu$ wide and 3.6 to 20 $\mu$ long. Ends pointed. The central part is frequently narrower and may show one or several definite bands. The organisms often lie in twos, with the adjacent ends rounded and the free ends pointed. The shorter lengths are not so wide, usually straight, seldom curved, while the longer are frequently more or less definitely curved or twisted. In cultures on sugar agar ten days old there are frequently threads of medium length with rounded ends. Long threads are seen in medium young colonies on sugar agar where also short and small spindle bacilli are present in lesser numbers. The long threads are 20 to 50 $\mu$ long. They are frequently twisted, of the same length throughout or occasionally narrower. In young broth cultures containing organs and in potato broth (Ori-Wrzosek) the organism is small, 3 to 5 $\mu$ long and .4 to .6 $\mu$ wide, rarely longer and thicker, regular, with sharply pointed ends. In eight-day sugar broth in an atmosphere of hydrogen short forms appear, usually 4 to 7 $\mu$ long. In hanging drops the organisms are structureless in young cultures. In old cultures one to two and sometimes more round and more refractile bodies appear in the protoplasm. Not motile. No spores.

**Staining Reactions.**—Young forms stain regularly, old forms irregularly. Stained best with dilute carbolfuchsin and Loeffler's methylene-blue. By Giemsa in one-half hour the protoplasm appears light bluish-red or light red. Gram-negative. Claudius-negative. In the bodies of the bacteria are one to two, occasionally more, round coccus-like bodies, staining intensely with various anilin dyes. In the long threads they are regularly separated from each other. Not present in young forms. Present in old fluid media. They stain intensely red with fuchsin, deep blue with methylene-blue, dark reddish with Giemsa. Not stained by Lugol solution in three to five minutes. In old cultures the staining is irregular, the bodies appearing barred, showing in their interior short, irregular, cylindrical areas with long pale stained areas between. Involution forms in old agar and potato cultures, the bacteria taking the stain with difficulty, and showing coarsely granular, irregular protoplasm. Long threads often show club-shaped swellings, protoplasm irregularly colored.

**Cultural Characters.**—Usually an obligate anaërobe. No growth on potato, protein-free media and peptone water. Growth in organ and potato broth aërobically at 37° C. The addition of sugar to agar brings about abundant development. Growth best in neutral or slightly alkaline media. In ascites sugar agar stabs (1 to 3) good growth in twenty-four hours along the stab at 37° C. with the exception of the upper 1.5 cm. The growth appears like a wide, opaque streak, pale brown, with many small points and lateral zones, cloudy and flocculent. Stab cultures in 1 per cent. sugar agar the same, except that the growth actually is visible in ten or twelve hours. Same type of growth on ordinary agar except slower. In sugar agar shake cultures the colonies are barely visible in twenty-four hours 1 cm. below the surface. In forty-eight hours the colonies are $\frac{1}{2}$ mm. in size. Under low power the colonies are round or oval with smooth edges, dark, with short fine felty prolongations. The colonies may also show a darker central area (nucleus) while the rest is finely granular, brownish-yellow, more transparent toward the periphery. No gas formation. In thinly seeded cultures colonies are 1 mm. in diameter in four days. On the surface of 1 per cent. sugar agar the bacillus grows in an atmosphere of hydrogen like a streptococcus. Colonies are dew-drop-like, $\frac{1}{2}$ to 1 mm. round, spherical, raised, grayish-white, and succulent. Under the hand lens they have smooth edges and are pale brown. In eight days they are thicker, 1 to 1.5 mm. in size, sometimes 2 mm., round, grayish-white, succulent, somewhat transparent. With a hand-lens they show 1 to 2 concentric rings, edges either smooth or finely indented. Thick white sediment in water of condensation. Grape sugar agar plates in an atmosphere of hydrogen at 37° C. show small colonies in five to six days, 1 mm. in diameter, grayish-white, dew-drop-like, spherical, raised, and moist. Under low power round, with smooth edges and a central nucleus, pale brownish-yellow, finely granular in the periphery, coarsely granular in the center. Colonies later 1.5 mm. in diameter.

In 1 per cent. sugar broth in hydrogen growth is poor after eight days at 37° C. as a whitish membranous sediment and clean fluid. In 1 per cent. gelatin covered with agar at

37° C. after five days there is a slight sediment, the gelatin is clear, but hardens on immersion in cold water. In organ broth after forty-eight hours at 37° C. slight blackening with an unpleasant odor. Broth clear, no gas.

*Potato Broth.*—Under aërobic conditions, in forty-eight hours at 37° C. good growth with a strong peculiar odor and a flocculent or cotton-like white sediment. Potato not changed. After six days more abundant sediment, fluid cloudy. In ascites broth (1 to 3) and in pure ascites fluid little growth in oxygen, the fluid remains clear with a very slight sediment. Little or no growth in atmosphere of $CO_2$.

The organism produces a foul unpleasant odor in all cultures, due in part at least to $H_2S$ and indol.

In sugar agar containing lead acetate in twenty-four hours the lower half of the stab shows diffuse pale brown color. On the fourth day a brownish zone appears around the stab. After ten to twelve days the entire stab is surrounded by a brownish-black zone, 1 mm. broad, failing in the upper 1.5 mm.

Indol is produced in sugar broth, organ broth, and potato broth in twenty-four to forty-eight hours.

No fermentation of grape sugar, lactose, fructose, saccharose, maltose, dextrin, glycerin, mannite, or urea.

Agar cultures containing litmus are rapidly decolorized. No hemolysis in blood agar.

**Non-pathogenic** for rabbits, guinea-pigs, and mice. Besson[2] found that the false membrane in cases of Vincent's angina when inoculated beneath the skin or into the muscles of laboratory animals produces abscesses and ulcerating necrosis in which fusiform organisms are present in abundance with other bacteria. Inoculation of pure cultures is generally without result.

1. Ozaki: Centralbl. f. Bakteriol. [etc.], 1 Abt., 1912, lxii, 76.
2. Besson: Practical bacteriology, microbiology and serum therapy. Hutchens' translation. 1913, p. 575.

### FUSIFORMIS TERMITIDIS Hoelling

Described by Hoelling[1] from the intestinal contents of termites. Spindle-shaped slightly pointed organisms with homogeneous protoplasm in young forms. The larger cells show a kind of alveolar structure. Some individuals have broad bands stretching across the middle of the cell staining deeply, while others have darkly staining oval masses in the center. These darkly staining portions of the cell Hoelling regards as true nuclei. They stain red or reddish-blue by nuclear dyes like Giemsa or polychrome methylene-blue, show a genuine structure, have a regular position in the cell and show all stages in division up to the formation of daughter nuclei. Not cultivated. The organisms apparently divide transversely. A similar organism was found in the blind gut of a mouse and named *Fusiformis muris*, as well as another species from fresh water which was not named.

Dobell[2] has observed spindle-shaped fusiform bacilli, 4 to 4.5 $\mu$ long, with sharply pointed ends, in the intestine of *Lacerta muralis*. Each cell contained a single darkly staining central mass regarded by Dobell as a nucleus. Fusiform bacilli all nucleated (?) were also found by Dobell in frogs, toads, newts (*Triton vulgaris*), and cockroaches (*S. orientalis*).

1. Hoelling: Arch. f. Protistenk., 1910, xix, 239.
2. Dobell: Quart. J. Micro. Sc., 1911, lvi, 395.

### FUSIFORM BACILLI IN APPENDICITIS

First cultivated by Veillon and Zuber[1] from the pus in appendicitis, the same organism being subsequently obtained by Grigoroff,[2] who regarded them as relatively frequent. In the pus they appear as a spindle-shaped rod with pointed end, two organisms often being united by their pointed extremities.

In cultures they present at first the same morphology, but soon produce granular swollen involution forms. Gram-negative and non-motile. Cultivated only anaërobically, best at 37° C. Growth slow at 22° C., appearing only after five to six days. Deep colonies in gelatin small, granular, grayish, circumscribed, with smooth borders. No liquefaction. Deep agar colonies in twenty-four hours at 37° C. small, whitish, under low power first grayish,

then brown and opaque. Majority lenticular. In the surface of agar an opaque thick humid layer develops like that of *Bacillus coli*, but more transparent.

Broth rapidly and densely clouded with a thick white sediment.

Some gas production, not enough to split agar stabs. Cultures fetid.

Cultures remain alive only five to six days.

Subcutaneous inoculation produces small abscesses in guinea-pigs and rabbits which heal spontaneously.

1. Veillon and Zuber: Arch. de méd expér. et d'anat. path., 1898, x, 517
2. Grigoroff: Thèse de Genève, 1905, p. 66.

## GENUS 4. PFEIFFERELLA Buchanan

Non-motile rods, slender, *Gram-negative*, staining poorly, sometimes forming threads and showing a tendency toward branching. Gelatin may be slowly liquefied. Do not ferment carbohydrates. Growth on potato characteristically honey-like.

Type species *Pfeifferella mallei* (Loeffler and Schütz) Buchanan.
*Pfeifferella pseudomallei* (Whitmore).

### PFEIFFERELLA MALLEI (Loeffler and Schütz) Buchanan

Discovered in glanders and established as the cause of the disease by Loeffler and Schütz (Struck[1]). This was confirmed almost immediately by Kitt[2] and by Weichselbaum.[3] Completely described by Loeffler[4] in 1886. Usually called *Bacillus mallei* or the "glanders bacillus."

**Morphology.**—Fine rods varying greatly in size with irregularly staining protoplasm, straight or curved, with rounded ends. They are shorter and thicker than tubercle bacilli. Often two rods appear joined by unstained intermediary substance. On acid agar twenty-four hours old they measure 0.3 to 0.5 by 0.7 to 2 $\mu$. Sometimes they are thicker and longer, measuring 0.7 by 1.5 to 3.5 $\mu$. They also appear almost coccoid, measuring 0.4 by 0.5 $\mu$ or as longer threads, measuring 0.4 by 7 to 8 $\mu$. In cultures on serum potato or neutral beef infusion kept eight to fourteen days at room temperature, the organisms when stained with methylene-blue look like micrococci with small blue masses lying in the body (Wladimoroff[5]).

Loeffler gives the length as 2 to 5 $\mu$, the breadth as 0.5 to 1 $\mu$.

In old broth cultures tangled threads of agglutinated organisms occur.

In the tissues of infected animals, as in the caseous lymph-glands of guinea-pigs or in the spleen of field-mice there are often individual bacilli shorter and thicker than the rest, the poles staining darker than the centers.

The glanders bacillus always shows a granular protoplasm demonstrated best by faint staining. Loeffler regards this as a degeneration while Bonome and Vivaldi[6] consider the light areas as vacuoles. When treated with Neisser's diphtheria stain (methylene-blue and vesuvin), dark spherical ovoid concavo-convex particles appear in a deep blue background, either on the ends or in the center (Marx). In old cultures the organisms involute and degenerate and films reveal chiefly small deeply-staining granules in unstained bacillary fragments.

According to Marx,[7] Galli-Valerio,[8] Conradi,[9] and Mayer[10] the glanders bacillus has clubbed ends and shows true branching. No spores. Non-motile.

**Staining Reactions.**—The glanders bacillus stains by all the anilin dyes, but with difficulty and loses the stain readily. It stains best by Loeffler's alkaline methylene-blue and by Kühne's carbol methylene-blue. It may also be demonstrated by Ziehl's carbolfuchsin, carbol thionin, carbol gentian-violet or Galli-Valerio's formalin methylene-blue. In the tissues it is brought out best by Loeffler's methylene-blue and Kühne's carbol methylene-blue. Gram-negative.

**Isolation.**—The glanders bacillus may be isolated without great difficulty from diseased tissues. It grows well under aërobic conditions, poorly in the depths of media or in an atmosphere of hydrogen. Optimum temperature 30° to 40° C. No growth above 43° C. or usually below 22° C. Old laboratory cultures sometimes grow at 20° C.

Growth better on media with slightly acid reaction, but growth occurs on neutral or alkaline media. The addition of 4 to 5 per cent. glycerin favors development. It grows well in broth made from the muscle of man, horse, sheep, beef, dog, rabbit, and hen with or without peptone. If the broth be overneutralized with HCl the growth is more vigorous.

**Cultural Characters.**—*Acid Agar 5 per cent.*—Colonies visible in twenty-four hours, usually very small, in forty-eight hours 0.5 mm. in diameter. They are round and regular, grow slowly, and measure 2 to 3 mm. in eight or nine days. Under low power they are round

Fig. 23.—*Bacillus mallei* from twenty-four-hour culture on acid agar, stained with 5 per cent. carbolfuchsin.

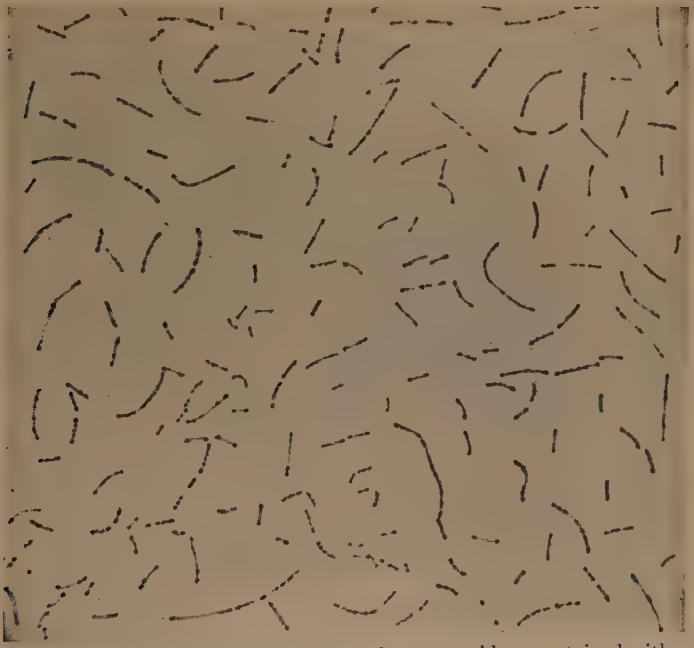

Fig. 24.—*Bacillus mallei* from forty-eight-hour culture on acid agar, stained with methylene-blue.

and regular with entire edges, usually with dense nuclei. Deep colonies punctiform under low-power biconvex disks.

*Acid Agar Slant.*—Slow growth, thin, scanty, and non-spreading in twenty-four hours. In two to three days the growth is more abundant and slightly granular. It increases in size and becomes more granular, reaching its maximum in ten to twelve days. Many strains have a pale straw-yellow color.

*Acid Agar Stab.*—Growth slow, appearing as a faint streak along the line of inoculation and a delicate surface expansion in forty-eight hours. The line growth increases slowly, while the surface expansion becomes abundant, somewhat elevated, straw yellow in color. The agar itself becomes very turbid.

*Glycerin Agar Not Neutralized.*—Wladimiroff regards this as the best medium. Colonies appear in forty-eight hours, flat dull white or gray. Older colonies are yellowish. At 37° C. the colonies become confluent, forming a wide expansion along the line of inoculation. This is 7 to 8 mm. broad in about a week. Water of condensation viscid with turbidity, precipitate, and superficial film.

*Dextrose Litmus Agar.*—Surface colonies appear in forty-eight hours, small, fine, $\frac{1}{2}$ mm. in diameter. They grow slowly and at the end of six to seven days are 1 to 2 mm. in diameter, regular, and uniform. Under low power they are round, regular, translucent, with entire edges. Often moruloid. Deep colonies pin-point under low power biconvex disks. The agar is at first acid in reaction, later, in eight to ten days, alkaline. In some strains the organisms fail to overcome the acidity.

*Dextrose Litmus Agar Slant.*—Growth appears in forty-eight hours as a thick, raised, whitish mass along the line of inoculation. In three days the growth is very thick, raised, moist, and slightly yellow. The agar has a highly acid reaction. Later the growth becomes still more abundant and in ten to twelve days the acidity is overcome and the agar becomes alkaline.

*Acid Glycerin Gelatin.*—Superficial colonies when young are delicate and transparent, showing faint zones. Under low power (60 diameters) they are irregular, roundish, wavy and indented, highly refractile. Older colonies are yellowish, especially in the center, with more definite markings. Deep colonies punctiform, under low power round or oval, coarsely granular in the center, streaked on the edges, peripheral zones strongly marked.

*Acid Glycerin Gelatin Stab.*—Discontinuous growth along line of stab, more abundant above than below, as a white or gray line without branching. On the surface a limited delicate transparent dull whitish or dirty-yellow expansion which may turn brown. A funnel-shaped depression develops at the surface due to drying, not to liquefaction.

At 37° C. the glanders bacillus develops in fluid gelatin as a white flocculent viscid mass.

*Blood-serum.*—Good growth on coagulated horse, sheep, or chicken serum, not so good on beef serum. Growth appears on the third day as yellowish drops with a slightly viscid consistency. After eight to ten days at 37° C. these drops lose their yellow transparency and become milky white from the deposition of small tiny crystals. Water of condensation viscid. Old cultures on beef serum yellowish-brown or brown. No liquefaction.

*Potato.*—This is the best medium for the glanders bacillus. Growth appears in forty-eight hours as a delicate yellowish transparent expansion. This growth increases in size, becomes homogeneous and thick, turns first amber colored, then reddish like the color of copper oxid. It may also be gray, coffee-brown, or chocolate-brown. It usually remains transparent like fluid honey. About the growth the potato itself develops a zone of color, greenish-yellow, greenish-blue, or green.

The transparency in potato cultures is due to the production of a viscid slimy substance. Sometimes when old cultures are transferred to potato they produce dry non-glistening folded expansions, straw-yellow or orange, like the growth of tubercle bacilli. Gradually viscid material develops between the folds and after a time the cultures become typical.

*Acid Broth.*—Fine cloudy turbidity in twenty-four to forty-eight hours, with a white viscid sediment. Broth does not clear. Grayish-white ring growth or viscid film, crawling up the sides of the glass. In old cultures a thick viscid coherent mass is deposited on the bottom, while the broth itself becomes thick, very viscid, first yellow to orange in color, then dark brown.

*Litmus Milk.*—No change in reaction, slow reduction of the litmus, late coagulation appearing in ten to twelve days.

Petruschky's milk whey becomes acid.

*Carbohydrates.*—Wladimiroff reports that the glanders bacillus produces acid from sugars. The present author's cultures fail to produce acid in dextrose saccharose or lactose. No production of indol or phenol.

**Resistance.**—The glanders bacillus survives four years in closed glycerin broth cultures (Wladimiroff). Dies in water in seventy-nine to eighty days (Finger). Survives eight and one-half months on glycerin gelatin (Schantyr), three to four months on glycerin agar (Loeffler, Straus). Survives but a short time on potato, transfers being necessary every three to four days. Destroyed by heat at 55° C. in ten minutes (Loeffler), at 61° C. in five minutes (Archa-

roff). Dried on silk threads dies usually in fourteen days, but may survive three months (Loeffler).

Not destroyed by liquid air (−185° to −190° C.) in five to ten minutes.

Destroyed rapidly by disinfectants, in one minute by sublimate 1 to 20,000, chlorid of lime 1 to 800, pure carbolic acid mixed with pure sulphuric acid 1 to 200, potassium hydrate 1 to 200, sodium hydrate 1 to 100, lysol 1 to 100, creolin and carbolic acid 3 to 100, milk of lime 4 to 100. It is also destroyed by concentrated alcoholic solutions of aniline dyes, methylene-blue, gentian-violet, malachite green, and fuchsin. Killed in five minutes by green soap.

**Pathogenic Action.**—Glanders occurs as a *natural disease* in horses, asses, mules, camels, and goats. Sheep, cattle, swine, and rats seem to be almost completely immune to both natural and artificial infection. Dogs and cats are sensitive to both types. Lions, tigers, and leopards in menageries develop glanders when fed on the flesh of glandered horses. Glandered animals exhibit an inflammation of the nasal mucosa and upper respiratory tract in acute cases, with an abundant serous, later seropurulent, nasal discharge. Ulceration of the nasal mucosa develops with an acute swelling of the neighboring lymph-nodes. When these break down they form deep ulcers and sinuses with an abundant discharge of pus. In chronic cases multiple nodules develop in the skin and there is a general enlargement of the lymphatic glands. These subcutaneous nodules may break down and ulcerate. In horses the chronic form of the disease is known as farcy. It may last for years and complete recovery rarely takes place. In fatal cases of glanders characteristic nodules are found in the internal organs. They consist of connective tissue with a diffuse leukocytic infiltration, the glanders bacilli lying in the center of the nodules. Natural infection takes place through the broken skin and through the nasal and buccal mucosa. In horses infection may take place through the injured mucosa of the digestive tract (Nocard[11]). The same channel is apparently followed in menagerie animals which develop the infection from feeding. In man glanders is not very uncommon in individuals who handle glandered horses. The infection penetrates the body usually through the injured skin where a nodule develops with a swelling of the adjacent tissues and a lymphangitis. Occasionally a papular eruption like variola appears (Zinsser[12]). Glanders infection in man is rapidly fatal, usually in eight to ten days, possibly from septicemia.

Cultures of the glanders bacillus are virulent to white and gray mice, field-mice, woods mice, guinea-pigs, rabbits, dogs, cats, and hedgehogs.

Among mice the field mouse (*Arvicola arvalis*) is most sensitive. Subcutaneous inoculation in mice produces death in two to eleven days. At autopsy there is an infiltration at the site of inoculation with lymphangitis and lymphadenitis, great enlargement of the spleen which is permeated with nodules (miliary abscesses). Nodules also occur in the liver and lungs. Occasionally a purulent arthritis of the joints of the feet may occur.

Rabbits are susceptible to subcutaneous inoculation in varying degree. In some instances the injection is followed by a local chancre-like ulcer which slowly heals by the formation of scar tissue. Or the animals may develop a true septicemia with glanders bacilli in the blood and organs. Finally they may develop the usual lesions characteristic of glanders, inflammation of the nasal mucosa, swelling of the glands, nodules in the lungs, liver, and spleen. The disease in rabbits lasts any time from five to one hundred and thirty days.

Guinea-pigs are especially sensitive to infection. After subcutaneous inoculation the animals may die in three to eight weeks, but death usually occurs in two to three months. At the site of injection a pasty tumor develops which is gradually converted into a chancre-like ulcer. The adjacent lymph-glands enlarge and may ulcerate. In some instances scar formation takes place and the ulcers heal. In other instances the ulcers persist and characteristic nodules develop in various areas in the skin and muscles. Suppurative inflammation may also occur in the joints of the feet. In male guinea-pigs an inflammation develops in the tunica vaginalis of the testicle with nodule formation, or in the part of the peritoneum which covers the musculus testis. This is known as Strauss's reaction.[13] Gradually a thick caseous pus is produced, the testicle is reddened and swollen, and open ulceration may follow. In about one-third of the cases an inflammation of the nasal mucosa occurs. At autopsy characteristic lesions with nodule formation are found in the lungs, liver, and greatly enlarged spleen. With intraperitoneal inoculation the infection is more acute, the animals dying in one to two weeks.

**Strauss's Reaction.**—This reaction is really a periorchitis and begins in two to three days, at which time the animal may be killed and the testicular tissue examined for the characteristic lesions, which also may be found in the spleen. Strauss's reaction is given by all true glanders bacilli, very rarely by other organisms like Nocard's Gram-positive bacillus of ulcerative lymphangitis, Kutscher's Gram-positive horse bacillus, and *Bacillus pseudomallei* of Whitmore. The diagnosis of glanders can be established by this method in forty-eight hours.

Cats are also very sensitive to the glanders bacillus and may be employed for rapid diagnosis (Lissitzyn,[14] Malzeff[15]). The animals are injected subcutaneously and killed in forty-eight hours. The organisms can be cultivated from the spleen and liver.

**Mallein.**—The glanders bacillus produces no true exotoxin. Its endotoxin is known as *mallein* and may be obtained by a variety of methods. It was discovered by Helmann[16] and

Koening, who made it from filtered aqueous and glycerin extracts of potato cultures. According to Nocard and Roux[17] mallein is best obtained by cultivating the organism in flasks containing 250 c.c. of 5 per cent. glycerin bouillon. After a month's cultivation at 35° C. the cultures are sterilized at 100° C. for thirty minutes, evaporated to one-tenth their original volume on a water-bath, and filtered through paper. This product is diluted ten times with 0.5 per cent. carbolic acid before use. The diagnostic dose for a horse is 0.25 c.c. of the undiluted fluid. Its subcutaneous introduction in glandered horses is followed by a rise in temperature to 104° to 106° F. and a local firm, hot, diffuse swelling 20 to 30 cm. in diameter. It is not poisonous to normal horses.

The production of mallein in the United States Department of Agriculture is carried out by growing the bacilli in glycerin broth for five weeks at 37° C. The cultures are boiled one hour and allowed to stand a week in a cool place. The supernatant fluid is decanted off, filtered through Berkefeld filters, evaporated to one-third its volume over a water-bath. The original volume is restored by the addition of 1 per cent. carbolic acid solution containing 10 per cent. glycerin.

Mallein is extremely resistant, withstanding temperatures of 120° C. and prolonged storage without loss of strength.

A precipitate obtained from filtrates of glanders cultures by the addition of absolute alcohol may also be used for diagnostic purposes. It is known as "morvin" or "dried mallein" and was first employed by de Schweinitz and Kilborne.[18]

Mallein can also be used for diagnosis by dropping it directly into the eye, usually diluted 1 to 4 or 1 to 10 (Vallée[19] and Martel[20]). A characteristic conjunctivitis develops in glandered horses, lasting up to thirty-six hours.

1. Struck: Deutsche med. Wchnschr., 1882, viii, 707.
2. Kitt: Jahrsb. d. Kgl. Central-Tierärzneischule, Munich, 1883–84.
3. Weichselbaum: Wien. med. Wchnschr., 1885, xxxv, 667; 700; 738; 766.
4. Löeffler: Arb. a. d. K. Gsndhtsamte, 1886, i, 46; 141.
5. Wladimiroff: *In:* Handbuch der pathog. Mikroorgan. (Kolle and Wassermann). 2. Aufl., 1913, v, 1063.
6. Bonome and Vivaldi: Deutsche med. Wchnschr., 1892, xviii, 985.
7. Marx: Centralbl. f. Bakteriol. [etc.], 1 Abt., 1899, xxv, 274.
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9. Conradi: Ztschr. f. Hyg., 1900, xxxiii, 161.
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12. Zinsser: Textbook of Bacteriology. 1922, p. 787.
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16. Helmann: Bote f. öffentl. Veter-Wesen (russ, 1891.
17. Nocard and Roux: Bull. Soc. centr. de méd. vét., 1892.
18. de Schweinitz and Kilborne: J. Comp. Med. and Vét. Arch., 1892, xiii, 643.
19. Vallée: Bull. Soc. centr. de méd. vét., 1907, lxi, 359.
20. Martel: Bull. Soc. centr. de méd. vét., 1907.

### PFEIFFERELLA PSEUDOMALLEI (Whitmore)

Described originally by Whitmore[1] in a glanders-like disease in man in which the lungs showed numerous patches of characteristic consolidation and subsequently found in a number of other cadavers. Named *Bacillus pseudomallei* by Whitmore. The disease caused by this organism was called *melioidosis* by Stanton and Fletcher[2] and it is regarded as a primary infection of rodents, rabbits, and guinea-pigs. The organism was later named *Bacterium Whitmori.*

**Morphology and Staining Reactions.**—Small actively motile bacteria about the size of the glanders bacillus. They show well-marked polar staining by Leishman and are non-acid-fast and Gram-negative. On salt agar the organisms grow in dense felted masses, made up of very long sinuous filments. Motility rapidly lost.

**Cultivated** easily on the usual laboratory media.

*Peptone Agar.*—Moist, translucent, slightly raised colonies in eight to ten hours. In forty-eight hours the colonies are thick, opaque, and cream-colored. After a month they are dry, wrinkled in the middle portion, brown in color with a tinge of pink.

*Salt Agar* (1½ to 2 per cent.).—Growth slow, appearing like a thin layer of white paint.

*Glycerin Agar* (3 to 5 per cent.).—Rapid and luxuriant development, the lower third of the culture acquiring a wrinkled appearance at the end of the second day. The wrinkling extends over the whole surface and at the end of a week the growth is heaped-up and rugose, not unlike the tubercle bacillus.

*Gelatin Streak.*—Slow development at 18° to 20° C. At the end of the third day a white streak is visible and beneath this the gelatin is slowly liquefied.

*Gelatin Stab.*—Faint white streak along line of stab in three days with a small white disk on the surface. By the fourth or fifth day the gelatin below this is liquefied. This later forms a small cup of liquid gelatin covered by a thick wrinkled pellicle. Along the line a white growth appears with extremely fine dots distributed in a radiating manner in the surrounding clear gelatin.

*Potato.*—Vigorous growth in twenty-four hours, cream-colored or light yellow. No discoloration of the potato and little spreading from the line of inoculation.

*Litmus Milk.*—No change in reaction in the first three days. Later the casein is precipitated, a thick white fluffy sediment collects at the bottom, the whey becomes pink and a violet scum forms on the surface, dotted with white spots.

*Carbohydrates.*—Glucose fermented with acid formation, also saccharose, lactose, mannite, and dulcite when freshly isolated strains are tested.

**Pathogenic** to guinea-pigs, wild rats, and rabbits. In guinea-pigs intraperitoneal inoculation of large doses leads rapidly to fatal septicemia. Small doses given subcutaneously or intraperitoneally produce a well-marked inflammation of the testicle (Strauss's reaction) in thirty-six hours and death occurs in four to five days. At the site of subcutaneous inoculation a thick caseous exudate develops. Infection can also be produced by feeding. The organisms are not virulent to horses (Stanton and Fletcher[3]). Serologically different strains of the organism form a homogeneous group to which some strains of *Bacillus mallei* are closely related.

1. Whitmore: J. Hyg., 1913, xiii, 1.
2. Stanton and Fletcher: Ann. Rep. Inst. Med. Res. Kuala Lumpur, 1919–1922.
———: Tr. Fourth Congress Far East. Assoc. Trop. Med., 1921, ii, 196.
3. Stanton and Fletcher: J. Hyg., 1925, xxiii, 347.

## E. ORDER EUBACTERIALES Buchanan

This order includes the forms usually termed the true bacteria, that is, those forms which are considered least differentiated and least specialized. The cell metabolism is not primarily bound up with hydrogen sulphid or other sulphur compounds, the cells in consequence containing neither sulphur granules nor bacterio-purpurin. The cells apparently do not possess a well-organized or well-differentiated nucleus. These organisms are usually minute and spherical, rod-shaped, or spiral, in most genera not producing true filaments, and rarely branching. The cells may occur singly, in chains, or other groupings. They may be motile by means of flagella, or non-motile, but are never notably flexuous. Cell multiplication occurs always by tranverse, never by longitudinal fission. Some genera produce endospores, particularly the rod-shaped types. Conidia not observed. Chlorophyll is absent, though the cells may be pigmented. The cells may be united into gelatinous masses, but never form motile pseudoplasmodia nor develop a highly specialized cyst-producing fruiting stage, such as is characteristic of the *Myxobacteriales*.

### Family I. Nitrobacteriaceae Buchanan

Organisms usually rod-shaped (sometimes nearly spherical in *Nitrosomonas* and possibly in *Azotobacter*). Cells motile or non-motile. Branched involution forms in *Rhizobium* and *Acetobacter*. Endospores never formed. Obligate aërobes, capable of securing growth energy by the direct oxidation of carbon, hydrogen, or nitrogen, or of simple compounds of these. Non-parasitic (except in genus *Rhizobium*)—usually water or earth forms.

#### Tribe I. Nitrobactereæ

Organisms deriving their life energy from oxidation of simple compounds of carbon and nitrogen (or of alcohol).

## GENUS 1. HYDROGENOMONAS Jensen

Monotrichic short rods capable of growing in the absence of organic matter, and securing growth energy by the oxidation of hydrogen (forming water). Kaserer (1905) who first described the organism states that his species will also grow well on a variety of organic substances.

Type species, *Hydrogenomonas pantotropa* (Kaserer) Jensen.
" *vitrea* Niklewski.
" *flava* Niklewski.

### HYDROGENOMONAS PANTOTROPHA (Kaserer) Jensen

First described by Kaserer[1] as a bacterial species, *Bacillus pantotrophus*, nourishing itself from carbonic acid obtained by the oxidation of hydrogen and at the same time growing upon nearly all the usual culture-media. Obtained by cultivating field earth in an atmosphere of carbon dioxid and hydrogen in a synthetic medium made up as follows:

| | Per cent. |
|---|---|
| $K_2HPO_4$, | 0.05 |
| $MgSO_4$, | 0.02 |
| $NH_4Cl$, | 0.1 |
| $NaHCO_3$, | 0.05 |
| Iron chlorid, traces | |

The ordinary bacterial species grow for the first few days, after which the hydrogen begins to disappear from the culture apparatus from oxidation. When a surface film is produced this is due to *Bacillus oligocarbophilus*, but other organisms are also present, including a liquefying species, probably *Bacillus methylicus* of Loew.

When the oxidation takes place without a surface film plates made from the material on gelatin give yellow colonies of an organism which has the capacity of oxidizing hydrogen. These colonies after five to six days are small and bright brownish-yellow or bright greenish-yellow and do not liquefy gelatin or do so very slowly. Superficial colonies are 4 to 5 mm. in size, deep colonies small and lens-shaped. Under low power the colonies are brownish-yellow and finely granular with smooth edges. This organism can be cultivated on ordinary media and has the following characteristics:

**Morphology.**—Short bacilli 0.4 to 0.5 $\mu$ wide by 1.2 to 1.5 $\mu$ long with round ends. Usually in pairs, occasionally in short chains.

*Motility.*—Active in young cultures. Flagella stained by Meyer's modification of Loeffler's flagella stain. Each organism possesses a single polar flagellum four to six times as long as the bacillus. Capsular substance abundant about the organisms easily stained by Johne's method. No spores. Easily stained. Old cultures show polar granules.

**Methods of Cultivation.**—Grows both aërobically and anaërobically. Grows best at 28° to 30° C.; less well at 37° C., not at 50° C. Slow growth at room temperature. Grows well on acid and alkaline media.

*Gelatin Plates.*—Colonies yellow, smooth, with concentric rings or greenish. No liquefaction. Growth good on acid gelatin made from peas, with occasional softening. The gelatin colonies appear after several days, the superficial reaching a diameter of 4 to 5 mm., the deep remaining small. Under low power the colonies are brownish-yellow, finely granular, always with smooth edges.

*Gelatin Streak.*—Limited non-spreading growth along line of inoculation.

*Gelatin Stab.*—Development only on the surface at the point of puncture as a yellow layer.

Cultures on *agar* like those on gelatin, but occasionally more greenish, often viscid.

*Broth.*—Somewhat viscid. Diffuse turbidity. Surface scum rare.

*Milk.*—No coagulation. Yellow growth on surface, a dirty flesh-colored mass in the depths.

*Potato.*—Yellow expansion, viscid, and refractile.

**Chemical Reactions.**—Oxidizes hydrogen to water in the presence of oxygen and an abundance of carbon dioxid. No formation of indol or hydrogen sulphid. No fermentation of sugar. Alkali production in meat extract sugar broth. No reduction of nitrates.

Kaserer finds that this organism oxidizes hydrogen, hastening the catalytic reduction of carbon dioxid to formaldehyd by hydrogen in such a way that it can utilize the formaldehyd as food material.

Widely distributed in field earth.

Other species described by Niklewski[2] are *Hydrogenomonas vitres* and *Hydrogenomonas flava.*

1. Kaserer: Centralbl. f. Bakteriol. [etc.], 2 Abt., 1906, xvi, 681.
2. Niklewski: Centralbl. f. Bakteriol. [etc.], 2 Abt., 1907–08, xx, 469.
———: Jahrb. f. wissensch. Bot., 1910, xlviii, 113.
———: [Abstr.] Centralbl. f. Bakteriol. [etc.], 2 Abt., 1910, xxviii, 514.

## GENUS 2. METHANOMONAS Jensen

Monotrichic short rods capable of growing in the absence of organic matter and securing growth energy by the oxidation of methane (forming carbon dioxid and water).

Type species, *Methanomonas methanica* (Söhngen) Jensen.

### METHANOMONAS METHANICA (Söhngen) Jensen

First isolated by Söhngen as *Bacillus methanicus*, an organism utilizing methane for its carbon requirements and as a source of energy. Methane is produced in large quantities by bacterial growth, but is present in the atmosphere only in small quantities. Since the oxidation of methane to carbon dioxid and water sets free a considerable quantity of energy it seemed reasonable to Söhngen to attempt to find micro-organisms which might so utilize it. Several plants and a special bacillus were shown to possess this property. Söhngen employed a special apparatus consisting of two Erlenmeyer flasks of 300 c.c. capacity of which one contained the following solution:

| | |
|---|---|
| Distilled water, | 100 c.c. |
| $K_2HPO_4$, | 0.05 |
| $MgNH_4PO_4$, 6 Aq., | 0.1 |
| $CaSO_4$, | 0.01 |

This was inoculated with garden earth, liquid dung, or pit water, the flask filled with oxygen and methane and placed at 30° to 37° C. At the end of two to four days a reddish-brown film of bacteria developed on the surface with a turbidity in the fluid below made up of bacteria, amebæ, and monads. At the end of a week the methane had almost entirely disappeared and a considerable quantity of carbon dioxid had been formed. The bacterial film was made up of a single kind of micro-organism which had the property of utilizing methane and which had the following characteristics:

**Morphology.**—Short thick rod measuring 1.5 to 2 $\mu$ in width by 2 to 3 $\mu$ in length. In older cultures the organism is shorter and looks more like a micrococcus. Motility in very young cultures due to a single flagellum.

Best obtained in pure cultures by cultivation in an atmosphere of $\frac{1}{3}$ $CH_4$ to $\frac{2}{3}$ air on washed agar to which the necessary organic salts are added. Transfers are made from the original film and at the end of two days pure colonies of *Bacillus methanicus* appear causing a slight turbidity in the agar.

Pure cultures produce a characteristic film on the synthetic medium in the apparatus described above. Since methane is the only source of carbon present it must serve for nourishment and energy. Part of the methane is utilized as material for the bacterial protoplasm, part as a source of energy. This is shown by the following analysis.

In the apparatus described above 102 c.c. culture fluid were placed and 225 c.c. $CH_4$ and 320.7 c.c. $O_2$ added. After fourteen days the mixture of gases had the following composition:

$CO_2$ = 78 c.c.
$CH_4$ = 0
$O_2$ = 172

The culture medium contained 21 c.c. $CO_2$.

The amount of methane thus utilized by the bacteria is very large. At the same time very considerable quantities of organic material accumulate in the culture. Thus 100 c.c. of the fluid in the above experiment used up 48.3 c.c. N/10 $KMnO_4$.

Söhngen: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xv, 513.

## GENUS 3. CARBOXYDOMONAS Jensen

Autotrophic rod-shaped cells capable of securing growth energy by the oxidation of carbon monoxid (forming carbon dioxid).

Type species:

*Carboxydomonas oligocarbophila* (Beijerinck and van Delden) Jensen.

### CARBOXYDOMONAS OLIGOCARBOPHILA (Beijerinck and van Delden) Jensen

Described by Beijerinck and van Delden[1] as *Bacillus oligocarbophilus*, a colorless organism which obtains its necessary carbon from an unknown carbon combination in the atmosphere and derives the energy necessary for its metabolism also from this source, both in the dark and light.

Isolated by the use of a synthetic medium with the following composition:

| | |
|---|---|
| Distilled water, | 100 |
| Disodium phosphate, | 0.01 |
| Potassium nitrate, | 0.01–0.1 |
| "Mineral solution," | 1 drop. |

The mineral solution is made up of:

| | | | |
|---|---|---|---|
| $MgSO_4$ | $0.7\ H_2O$ | = | 6 mg. |
| $MnSO_4$ | $0.4\ H_2O$ | = | 0.05 mg. |
| $FeCl_3$ | $0.3\ H_2O$ | = | 0.05 mg. |

This fluid is inoculated with garden earth and under the usual aërobic conditions cultivated in the dark at 23° to 25° C. At the end of two to three weeks the fluid is covered with a thin, snow-white, very dry, hard network which microscopically consists of very small bacteria embedded in a slimy mucinous substance. The bacterial film develops steadily and a considerable amount of organically bound carbon accumulates in the fluid below as shown by the increase in bacteria and also by testing with permanganate.

From the surface film on this synthetic media pure cultures of the organisms known as *Bacillus oligocarbophilus* can be obtained by the use of special solid media. Development is favored by the presence of potassium, phosphorus, and magnesium and nitrates. Nitrites or ammonia salts are necessary as a source of nitrogen, but the organism is not a nitrifying species.

The organism does not grow on ordinary culture media because these contain too much organic carbon compounds. Cultivation is successful on agar which is thoroughly washed to rid it of organic salts and then made up into the following special medium:

| | |
|---|---|
| Distilled water, | 100 |
| Agar, | 1.5 |
| $K_2HPO_4$, | 0.01 |
| $KNO_3$ (or $NH_4Cl$), | 0.01 |

This is poured on a plate and inoculated with cultures of the organism from an original film. The accompanying bacterial contaminations, which are always present originally, use up the soluble carbon compounds not entirely removed from the agar by washing, after which *Bacillus oligocarbophilus* begins to develop. This is usually after fourteen days. The other organisms cease to develop because of their inability to utilize the atmospheric carbon compound, *Bacillus oligocarbophilus* being the only organism capable of so doing. The nitrifying ferments, present also in the original preparation when ammonia or nitrites are used as a source of nitrogen instead of nitrates, develop till their colonies are about 1 mm. in size and then stop. The colonies of *Bacillus oligocarbophilus* are 1 cm. or larger and can easily be transferred and obtained in pure culture. These colonies are thin snow-white or rose-colored, very dry, covering the entire agar and resembling the original film on the first cultures.

The organism also grows well on a medium made up of water glass (sodium or potassium silicate) diluted with water and treated with normal hydrochloric acid. The reaction should not be adjusted to complete neutralization since the clotting of the silica is promoted by an alkaline reaction. Since the plates contract markedly with a high percentage of silicic acid the original dilution with water should be sufficient to prevent this contraction. For example, 5 c.c. water glass is diluted with 25 c.c. water mixed with 10 c.c. normal hydrochloric acid and poured into a glass beaker. This mixture of diluted water glass and acid gradually clots

into a firm mass which should then be freed from chlorids by washing with tap water and then with boiled water. It is then covered with the same solution of salts as used above. By gentle heating from the under side the water in the plates can easily be vaporized and the plates then have a hard dry surface which can be sterilized by passing through the flame of a Bunsen burner. The nitrifying bacteria as well as *Bacillus oligocarbophilus* grow well on this medium. If the diluted water glass is mixed with chalk, magnesium carbonate, or ammonia magnesium phosphate pure snow-white plates can easily be secured. It is especially important that the jelly should contain no organic substances since even pieces of cork stopper which may drop in accidentally can ruin the experiment.

In the pure cultures on solid media the organisms are small, thin, short rods always without motility, about 0.5 $\mu$ thick and 0.5 to 4 $\mu$ long. They are usually from 0.7 to 1 $\mu$ long. The organisms are difficult to find in the mass of culture material, since the mucinous cellulose walls of the bacteria form the greater portion. The protein substance of the bacterial body is present only in small quantity.

By a number of carefully conducted experiments it was now shown by the authors that *Bacillus oligocarbophilus* when cultivated on these special media containing the above salts can utilize certain organic carbon compounds in the atmosphere, but not the carbon dioxid, either free or in combination. It was subsequently shown by Kaserer[2] that this species utilizes atmospheric carbon monoxid. In symbiosis with other bacteria (*e. g.*, *Bacillus methylicus* Loew) it can also oxidize hydrogen. This reaction apparently takes place in such a way that the catalytic reduction of carbonic acid to carbon monoxid by means of the hydrogen is so hastened that the organism can assimilate the carbon oxid as nutritive material.

1. Beijerinck and van Delden: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1903, x, 33.
2. Kaserer: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xvi, 681.

## GENUS 4. ACETOBACTER Fuhrmann

Cells rod-shaped, frequently in chains, non-motile. Cells grow usually on the surface of alcoholic solutions as obligate aërobes, securing growth energy by the oxidation of alcohol to acetic acid. Also capable of utilizing certain other carbonaceous compounds, as sugar and acetic acid. Elongated, filamentous, club-shaped, swollen, and even branched cells may occur as involution forms.

Type species *Acetobacter aceti* (Thompson) Com.
" *pasteurianum* (Zopf).
" *xylinum* (Brown).
" *acetosum* (Henneberg).

Thermophilic:
*Bacillus Zeidleri* or *Bacterium Linderi* Zeidler.

### ACETOBACTER ACETI (Thompson) Com.

Originally investigated by Kützing[1] and called *Ulvina aceti*, by Thompson[2] and named *Mycoderma aceti*. A species, possibly the same, was later investigated by Pasteur[3] in 1868. The name *Bacterium aceti* was given by Zopf[4] in 1885 and *Bacterium aceti* (Kützing) Zopf by Migula[5] in 1900. The term "*Acetobacter*" was employed by Fuhrman[6] in 1905 and adopted by the Committee as a genus name for the acetic acid bacteria. Especially studied by Hansen.[7] Present description from Migula.

**Morphology.**—Short rods 0.7 to 1 $\mu$ thick and 1.5 to 2.5 $\mu$ long, usually in short or long chains. Greatest variation in size and shape. Long, bent, swollen, and occasionally retracted involution forms common even in young cultures. These may be five to eight times the thickness of the ordinary rod and twenty times the length. According to Fuhrman true branching may also occur. No spores. No motility. The organisms stain yellow with iodin.

**Methods of Cultivation.**—Grows best in such materials as "double beer" containing about 1 per cent. alcohol after sterilization. Here it forms in twenty-four hours at 34° C. a mucinous viscid uniform film which tends to become veined. The fluid below remains clear even when transferred to lower temperature. Microscopically this film is made up of peculiar organisms often like an hour glass in shape, and often joined together in long articulated chains, seldom in long threads. When kept at 40° to 40½° C. long thin non-articulated threads are formed.

On *beerwort gelatin* at 25° C. colonies develop in four days. They are round, raised, with entire edges, seldom star shaped, viscid or mucinous by direct light, bluish by transmitted light. These colonies consist of short individual rods without many articulated threads.

If a drop of culture material be placed on a thick layer of *beerwort gelatin* and the preparation be kept at 25° C. a wide-spread colony develops. After about eighteen days this colony forms a kind of rosette with toothed or spiked edges.

On *meat extract peptone gelatin* the colonies are much the same, but are surrounded by milky zones separated from the colonies by clear rings.

On *double beer gelatin* similar transfers give rise to a round dry spreading colony with entire edges which may be slightly heaped up on the surface. After eighteen to twenty days this also takes on a rosette formation.

On *double beer* this organism grows at temperatures between 4° and 42° C. It is widely distributed in various kinds of beer, in atmospheric dust, and in water. It can be cultivated on *wort gelatin*, but not generally on ordinary *meat infusion peptone gelatin*.

**Chemical Reactions.**—*Acetobacter aceti* is the typical representation of a group of acetic acid bacteria which have the especial ability to develop aërobically upon the surface of alcoholic solutions, securing their growth energy by the oxidation of the alcohol to acetic acid. They are also capable of utilizing other carbon compounds such as sugar and acetic acid.

1. Kützing: J. f. prakt. Chem., 1837, ii, 385.
2. Thompson: Ann. d. Chem. u. Pharm., 1852, lxxxviii, 89.
3. Pasteur: Etudes sur le vinaigre. Paris, 1868.
4. Zopf: Spaltpilze. 3. Aufl., 1885, p. 62.
5. Migula: System der Bakterien. 1900, ii, 399.
6. Fuhrman: Beiheft zum Bot. Centralbl., 1905, xix, 1.
7. Hansen: Meddelelser fra Carlsberg Laboratoriet. 1894, iii.

### ACETOBACTER PASTEURIANUM (Zopf)

An organism closely related to *Acetobacter aceti* is differentiated from it by Hansen as *Mycoderma Pasteurianum*. This was named *Bacterium Pasteurianum* by Zopf and later investigated carefully by Hansen.

**Morphology.**—Organisms similar to *Acetobacter aceti*, but a trifle larger. Differentiated by their staining blue with iodin instead of yellow.

**Methods of Cultivation.**—On *double beer* with 1 per cent. alcohol (after sterilization) this organism at 34° C. forms a very superficial scum in twenty-four hours which is dry on the surface and forms many wrinkles or folds. The fluid below remains clear even when transferred to room temperature. Microscopically this film is made up of cells larger and thicker than those of *Acetobacter aceti*, also arranged in chains. At 40° to 40½° C. it forms similar long non-articulated threads, but somewhat thicker.

Colonies on *beerwort gelatin* develop at 25° C. They are smaller than those of *Acetobacter aceti*, and consist of typical chains. On *meat infusion gelatin* the colonies are much the same as those of the former species. On the surface of a thick layer of *beerwort gelatin* transfers give rise after eighteen days to a colony with smooth or slightly toothed edges. On *double beer gelatin* colonies appear in two to three days like those of *Acetobacter aceti*, dry on the surface and folded after eighteen to twenty days.

Growth at 5° to 6° C. and at 42° C. Distribution the same as that of *Acetobacter aceti*. Also forms acetic acid from alcohol.

### ACETOBACTER XYLINUM (Brown)

Described originally by Brown[1] and investigated subsequently by Henneberg.[2]

Non-motile non-sporulating rods forming a very thick (even 1 cm.) leathery scum on fluids. The organisms are like *Acetobacter aceti*, and give the cellulose reaction with iodin and sulphuric acid. According to Henneberg this species oxidizes ordinary alcohol, prophyl alcohol, and dextrose. The vinegar has a pungent odor and is quickly consumed.

Optimum temperature 33° C.

Maximum alcohol quantity in which oxidation occurs 6 per cent. Maximum amount of vinegar 4 per cent. Found in wine, in rapidly souring beer, and in malt acetic acid.

1. Brown: Chemical Society, 1886, i, 432. *Ibid.*, 1887, i, 643.
———: Ber. d. deutsch. chem. Gesellsch., xix, 463.
2. Henneberg: Zur Unterscheiding der Essigbakterien deutsche Essigindustrie, 1898, Jahr. II, No. 14, 115.

### ACETOBACTER ACETOSUM (Henneberg)

Found by Henneberg in beer.

**Morphology.**—Long parallel-disposed threads made up of cylindrical rods about 0.4 to 0.8 $\mu$ wide and 1 $\mu$ long. Much like *Acetobacter pasteurianum* in morphology. Similar involution forms on beer and dextrose agar.

Non-motile.

Not colored blue with iodin.

**Cultural Characters.**—*Gelatin* colonies like those of *Bacillus oxydans* (*Bacterium oxydans* of Henneberg). Forms firm regular, smooth, white scums on fluid media, which fold as they get older. The fluid remains clear. On a 6 per cent. *dextrose agar* solution forms a scum made up of isolated islands which on shaking do not sink to the bottom. Oxidizes ordinary alcohol, propyl alcohol, glycol, arabinose, galactose, and dextrose.

Optimum temperature 28° C. Grows at 36° C. Forms vinegar containing acetic ether. Maximum alcohol content in which oxidation takes place 11 per cent. Maximum vinegar content 6.6 per cent. Found in top beer.

Henneberg: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1897, iii, 223. *Ibid.*, 1898, iv, 14.

### ACETOBACTER ZEIDLERI

Described originally by Zeidler[1] as a thermophile producing acetic acid. Known also as *Acetobacter Linderi* and *Bacterium Zeidleri.*

**Morphology.**—Rods like those originally described by Cohn in his thermophilic bacteria. Produces involution forms quickly even in young cultures, especially in beerwort. Involution forms common in older wort gelatin and beer cultures, according to Hansen[2] both in the sediment and in the film along the wall of the container. They appear as long and short chains and rods. They are especially abundant in unfavorable media as hop beer wort with 12 per cent. alcohol.

**Motility.**—Characteristic motility in beerwort cultures up to the formation of an acidity of 4 to 5 c.c. In beer cultures motility ceases after an acidity of 30 to 35 c.c. is reached. This acidity is reckoned in the number of cubic centimeters N/10 NaOH solution which are necessary to neutralize 10 c.c. of the fluid tested. Somewhat slower motility persists in wine till a film is formed on the surface. Motility lasts for a week in culture media in which no acid is formed.

**Cultural Characters.**—Forms visible colonies in three days in *wort gelatin* and *beer gelatin.* These are circular, somewhat granular in the middle, brownish-yellow, with a bluish shimmer on the smooth edges. Superficial colonies develop until they appear as light yellow drops, heart-shaped or pear-shaped. No liquefaction. In *beer gelatin* a pungent odor of vinegar is given off. Surface cultures in *gelatin* are scanty. They form opalescent transparent expansions, white in the middle, with smooth edges, becoming confluent after a week. No definite liquefaction of the gelatin.

On *meat infusion gelatin* a thin pale yellow surface expansion appears, surrounded by a milky iridescent zone. In stab cultures only a slight development along line of inoculation. Later only a surface expansion is visible.

No growth on *potato.*

In fluid cultures like *hop beerwort* this organism forms a cloudy turbidity on the surface, later extending through the entire fluid. This has a dirty-yellow-brown color and gives off an odor like celery. On the surface it forms a film of small easily dislocated islands without slimy consistency, with a white bluish-shimmering ring growth. After the cessation of growth a loose brownish sediment is deposited, the supernatant fluid is clear and strongly opalescent.

In *beer* the growth is slower, appearing about twenty-four hours later than in wort. The surface islands are better developed, but a complete surface film is not formed. In *wine* it forms a very soft easily broken film made up of individually disposed rods. After a few days these rods grow out into long threads and the film is firmer, but not a true surface scum. If the vessel containing it is not disturbed the wine remains clear.

These organisms also develop in nutrient solutions containing only ammonia salts as a source of energy. They develop likewise in a pure 1 per cent. dextrin solution with a 3.42° C. rotation. This later gives a definite copper precipitate with Fehling's solution. The resulting fluid is always turbid.

1. Ziedler: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1896, ii, 730.
2. Hansen: Recherches sur les bactéries acetifiantes.

## GENUS 5. NITROSOMONAS Winogradsky

Includes *Nitrosococcus* Winogradsky

Cells rod-shaped or spherical, motile or non-motile, if motile with polar flagella. Capable of securing growth energy by the oxidation of ammonia to nitrites. Growth on media containing organic substances scanty or absent.

Type species, *Nitrosomonas europæa* Winogradsky.
" *javaniensis* Winogradsky.
*Nitrosococcus* of Winogradsky.

### NITROSOMONAS EUROPŒA Winogradsky

Originally classified by Winogradsky[1] as a Nitromonas. Later given the name *Nitrosomonas europæa*.[2] Same as *Pseudomonas europæa* (Winogradsky) Migula.[3]

Forms nitrites from ammonia salts.

Found in earth from various parts of Europe, Zurich in Switzerland, and Gennevilliers in France. Probably the same as the nitrifying ferments of the Franklands[4] and possibly the same as those obtained by Jordan and Richards.[5] Cultivated in solutions containing ammonium sulphate 0.2 to 0.25 per cent. and other salts to which magnesium carbonate is added. Often 0.2 per cent. sea salt is added also. The fluid is first clear and after about seven days becomes opalescent and turbid. At this stage the organisms appear as motile free cells or "monads," measuring 0.9 to 1 $\mu$ by 1.2 to 1.8 $\mu$. They may be isolated in small groups or in chains. In another twenty-four to forty-eight hours the turbidity begins to disappear. Nitrites are being formed and the ammonia used up. Then a gelatinous material forms about the precipitate of carbonate which microscopically consists of masses of zoöglœa. The development of the organism is now complete and the nitrification at an end. The bacterial cells are surrounded by a gelatinous membrane or capsule and are slowly motile. Flagella may be demonstrated by the use of Loeffler's stain, adding 10 to 15 drops of 1 per cent. soda solution to the mordant. The flagella appear as short single polar spirals somewhat larger than the rods.

Cultivated on *silicic acid jelly* the colonies appear about the fourth day, round and refractile, with black edges. In seven to eight days they attain a diameter of 40 to 60 $\mu$, are compact, clear or brownish or brownish-black. The surface colonies remain round and the deep colonies become angular. In the early stages the colonies consist of ellipsoid resting forms, 0.9 to 1 by 1.1 to 1.8 $\mu$ in size. They often resemble spindles with blunt ends and rarely are united in short chains of three to four elements. Later the colonies are made up of zoöglœa masses. After ten to fourteen days the colonies are surrounded by clear colorless masses, refractile, angular or pointed, and slightly viscid. These masses are made up of motile monads. Liquid cultures containing monads give rise to clear colonies. Cultures containing zoöglœa give rise to dark colonies. Can be cultivated also on agar washed free from soluble organic substances to which the suitable salts are added.

The organism studied by Jordan and Richards was cultivated in the following medium:

| | |
|---|---|
| Ammonium chlorid, | 1.9072 gr. |
| Sodium carbonate, | 3.7842 gr. |
| Sodium phosphate, | 0.2000 gr. |
| Potassium sulphate, | 0.2000 gr. |

These salts were dissolved in enough distilled water so that the solution contained 100 parts nitrogen per 100,000 parts and 2 equivalents of alkali. Ten c.c. of this solution was mixed with a liter of redistilled water. This organism was a short oval bacillus 0.8 to 0.9 $\mu$ broad and 1.1 to 1.7 $\mu$ long. Irregular zoöglœa were associated in jelly like masses at the bottom of the flasks containing the medium. No motility demonstrated. Not surely determined to be identical with Winogradsky's form.

1. Winogradsky: Ann. de l'Inst. Patetur, 1890, iv, 257.
2. Winogradsky: Arch. d. sc. biol., 1892, i, 87.
3. Migula: System der Bakterien. 1900, ii, 954.
4. Frankland (G.) and Frankland (P.): Philosophical Transactions, 1890, clxxxi.
———: Proc. Roy. Soc. Lond., 1890, xlvii, 296.
5. Jordan and Richards: Report on Purification of Sewage and Water, Mass. State Dep. Health, 1890, ii, 864.

### NITROSOMONAS JAVANIENSIS Winogradsky

Originally described by Winogradsky.[1]

Known also as *Pseudomonas javaniensis* (Winogradsky) Migula.[2]

Sinilar in properties to *Nitrosomonas europæa*. Occurs as swarming cells and zoöglœa, which are often so compact that it is difficult to find the individual cells. These are almost spherical, 0.5 to 0.6 $\mu$ in diameter. Motility slow, due to extremely long polar flagella often 30 $\mu$ long, stained by Loeffler's method, except for the addition of 8 drops of 1 per cent. soda solution to 10 c.c. of the mordant.

Methods of cultivation same as those for *Nitrosomonas europæa* except that the dark colonies are coffee-brown.

Found in earth from Java.

1. Winogradsky: Ann. de l'Inst. Pasteur, 1891, v, 92.
2. Migula: System der Bakterien. 1900, ii, 954.

### Nitrosococcus of Winogradsky

Described by Winogradsky.[1] Known also as *Micrococcus nitrosus* (Winogradsky) Migula.[2]

**Morphology.**—Large cocci, 1.5 to 1.7 $\mu$ in diameter, enclosed in a thick mucinous envelope which cannot be stained and is invisible after drying.

No motility demonstrated.

Forms large beautiful colonies on silicic-acid jelly. Superficial colonies have the appearance of small drops of turbid yellow fluid. Under low power they show coarse granules or pearls. Even with a magnification of a hundred diameters the individual cocci are visible as free cells lying in fluid masses inside the jelly-like membranes.

Found in samples of earth from Quito.

A larger coccus with similar properties, 2 $\mu$ in diameter, was found in earth from Campinas and a smaller one in earth from Melbourne.

1. Winogradsky: Ann. de l'Inst. Pasteur, 1891, v, 577.
———: Arch. d. sc. biol., 1892, i, 87.
2. Migula: System der Bakterien. 1900, ii, 194.

## GENUS 6. NITROBACTER Winogradsky

Cells rod-shaped, non-motile, not growing readily on organic media or in the presence of ammonia. Cells capable of securing growth energy by the oxidation of nitrites to nitrates.

Type species *Nitrobacter Winogradsky* Com.

### Nitrobacter winogradskyi Com.

Found first by Winogradsky.[1] Known also as *Bacterium nitrobacter* (Winogradsky) Migula.[2] Isolated from the soil of Quito.

**Morphology.**—Small thick rods, elongated, cylindrical, or oval, measuring 0.5 by 2 to 3 $\mu$, embedded in a mucinous material. Non-motile. Stains with difficulty. Can be demonstrated by staining with malachite green, gentian, or fuchsin, and then washing in hot water at 50° to 60° C. Forms nitrates from nitrites.

According to Kruse this form is small, measuring 0.2 to 0.25 $\mu$ by 0.5 $\mu$ in size. It forms lens-shaped lamelliform yellowish-gray colonies on silicic acid jelly and thin films in fluid media five to six weeks old. No turbidity. The film adheres to the walls and bottom of tubes and on shaking rises and appears as small floccules in the fluid.

1. Winogradsky: Ann. de l'Inst. Pasteur, 1891, v, 577.
2. Migula: System der Bakterien. 1900, ii, 514.

## Tribe 2. Azotobactereæ Com.

### Nitrogen-fixing organisms

## GENUS 7. AZOTOBACTER Beijerinck

Relatively large rods, or even cocci, sometimes almost yeast-like in appearance, dependent primarily for growth energy upon the oxidation of carbohydrates. Motile or non-motile; when motile, with tuft of polar flagella. Obligate aërobes, usually growing in a film upon the surface of the culture

medium. Capable of fixing atmospheric nitrogen when grown in solutions containing carbohydrates and deficient in combined nitrogen.

Type species, *Azotobacter chroococcum* Beijerinck.
" *agilis* Beijerinck.

### AZOTOBACTER CHROOCOCCUM Beijerinck

Cultivated by Beijerinck as an oligonitrophile capable of assimilating free atmospheric nitrogen. Found in garden earth and in the canal water at Delft. Isolated by means of aërobic culture media in thin layers in large Erlenmeyer flasks, which do not favor or permit butyric acid fermentation due to *Clostridium pasteurianum* of Winogradsky. For this purpose 2 to 10 per cent. solution of mannite serves $\frac{1}{2}$ per cent. calcium potassium or sodium propionate. In such solutions ordinary bacteria requiring much nitrogen (polynitrophyles) will not develop. The following medium inoculated with garden earth gives good results:

| | |
|---|---|
| Tap water, | 100 g. |
| Mannite | 2 g. |
| $K_2HPO_4$, | 0.02 g. |

At the end of two to three days a scum develops on the surface of this nitrogen-poor medium made up of peculiar large bacterial cells. This film develops like a mold and contains these bacteria, as well as amebæ, monads, infusoria, and other bacteria. With 0.5 per cent. calcium propionate as a source of carbon instead of mannite the surface scum consists almost entirely of *Azotobacter chroococcum*. Similar results may be obtained with glucose, levulose, galactose, saccharose, and maltose, and more slowly with 2 to 3 per cent. glycerin. The organism oxidizes propionates, butyrates, lactates, malates, succinates, acetates, and citrates with the production of carbon dioxid and water. Tartrates and formates not oxidized.

**Morphology.**—In the original surface membranes and in cultures the organisms appear as very thick short rods, 4 $\mu$ thick and 5 to 7 $\mu$ long, with rounded ends, often arranged as giant diplococci. Usually non-motile, a few cells showing leisurely movements. The cells are surrounded by a thick mucinous capsule. In young cultures vacuoles appear in the organisms and in mannite solutions they contain fat. In older preparations the cell membranes become brown and almost black, and the cells are arranged in sarcina-like packets. The brown pigment is insoluble in water, alcohol, ether, chloroform, and carbon bisulphid, and soluble with difficulty in alkalies.

In later cultures the organisms look like small micrococci, arranged in large sarcina-like packets.

Involution forms very common, especially in the original membranes. These may appear as giant-cells, 10 to 15 $\mu$ in diameter, and like amebæ and yeast cells. Cilia stained, but with difficulty on the motile form because of the mucinous material about the organism. They are usually polar, single or several, and may be on the sides, but near the ends. Non-motile forms show no cilia.

**Methods of Cultivation.**—Pure cultures obtained easily by transferring to media of the following composition:

| | |
|---|---|
| Distilled water, | 100 g. |
| Mannite, | 2 g. |
| $K_2HPO_4$, | 0.02 g. |
| Agar, | 2 g. |

The 2 per cent. agar contains the other mineral salts necessary for development. Grown at 30° C. colonies appear in twenty-four hours, paste-like, and are easily distinguished from the watery transparent colonies of the nitrophiles. In a few days the accompanying bacteria cease to grow, while the colonies of *Azotobacter chroococcum* continue to develop.

Pure cultures can be cultivated on all sorts of media, such as gelatin made of decoctions of pea-leaves with 2 per cent. saccharose, on 2 per cent. agar in tap water with 4 per cent. glucose, and on ordinary meat extract gelatin. On the latter the growth is moderate and a slight liquefaction sometimes occurs.

In fluid media pure cultures grow well when small quantities of all sorts of nitrogenous substances are added. Nitrates are especially well assimilated in a concentration of 1 gram per liter. Excellent development in the following medium:

| | |
|---|---|
| Tap water, | 100 g. |
| Mannite, | 2–10 g. |
| $K_2HPO_4$, | 0.02 g. |
| $KNO_3$, | 0.1 g. |

Ammonia salts assimilated as in the following material:

| | |
|---|---|
| Tap water, | 100 g. |
| Glucose, | 2 g. |
| $K_2HPO_4$, | 0.02 g. |
| $(N_4H)2HPO_4$, | 0.02 g. |

Asparagin is also assimilated, but not peptone.

Beijerinck: Centralbl. f. Baklteriol. [etc.], 2. Abt., 1901, vii, 561.

### AZOTOBACTER AGILIS Beijerinck

Found in canal water at Delft by Beijerinck in association with *Azotobacter chroococcum*. Not found in garden earth. Isolated easily by the use of the following solution:

| | |
|---|---|
| Pit water, | 100 g. |
| Mannite | 2 g. |
| $K_2HPO_4$ | 0.02 g. |

This is used in thin layers in Erlenmeyer flasks and kept at 25° to 30° C. In three to seven days a membrane develops which consists of a culture of this organism, more or less pure. The viscidity of the membrane is less than that of *Azotobacter chroococcum*, the membrane is looser and microscopically the organisms are usually isolated.

*Azotobacter agilis* belongs to the "meso-aërophiles"; that is, it develops best not in the meniscus, but some distance from it. Like the spirilla it seeks a medium oxygen pressure. It has a greater need of oxygen than the spirilla, however, and thus corresponds more to the macro-aërophiles.

This organism assimilates various sugars including saccharose in 2 per cent. solution. It also assimilates ½ per cent. calcium lactate. Propionates and succinates also serve as a source of carbon material. These two latter, however, favor the development of amebas and monads too much. Glucose and levulose are more easily assimilated by pure cultures than mannite.

Pure cultures easily obtained on the following medium:

| | |
|---|---|
| Distilled water, | 100 g. |
| Agar, | 2 g. |
| Glucose, | 2 g. |
| $K_2HPO_4$ | 0.02 g. |

The other necessary mineral salts are provided in the agar. Small colonies develop in twenty-four hours and can easily be recognized by the microscopic characters of the elements forming them.

If ½ per cent. calcium propionate be substituted for the glucose the colonies are surrounded by a wide-spread diffusion area of a greenish-yellow pigment like that of the fluorescent bacteria.

Pure cultures grow well on various media. Growth is abundant on *meat-infusion gelatin*. A white precipitate forms in the region of the streak and the reaction becomes alkaline. No liquefaction. Grows also on *meat-infusion agar*. In *meat-infusion gelatin* containing 2 per cent. *sugar, e. g., saccharose,* the cultures form a lot of mucinous viscid material. This is due to the thick mucinous cell walls of the individual elements. On *glucose agar* and on media poor in nitrogen old cultures of *Azotobacter agilis* produce a diffusible dark violet pigment. Pure cultures assimilate glucose and levulose more easily than mannite.

The organisms are motile, especially from infusion gelatin or infusion agar. This is due to bunches of 6 to 10 flagella attached to both poles, but often stuck to the sides of the organism.

Beijerinck: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1901, vii, 561.

## GENUS 8. RHIZOBIUM Frank

Minute rods, motile when young. Involution forms abundant and characteristic when grown under suitable conditions. Obligate aërobes, capable of fixing atmospheric nitrogen when grown in the presence of carbohydrates

in the absence of compounds of nitrogen. Produce nodules upon the roots of leguminous plants.

Note.—While *Phytomyxa* Schröter has priority over *Rhizobium* Frank, the Committee recommended the adoption of the latter better-known generic name, to avoid confusion.

Type species *Rhizobium leguminosarum* Frank.

#### RHIZOBIUM LEGUMINOSARUM Frank

First described by Beijerinck[1] in 1888 as *Bacillus radicicola.*

**Morphology.**—Rods 0.9 $\mu$ thick and 3 to 4 $\mu$ long with slightly rounded ends, usually in single elements. Swarming forms 0.18 $\mu$ thick and 0.9 $\mu$ long described by Beijerinck. Peculiar bacteroids appear in cultures as well as in the root tubercules (Kruse). Slowly motile, in artificial cultures often non-motile. No spores.

**Cultural Reactions.**—Scanty growth on ordinary *gelatin.* Growth best on a medium containing 7 per cent. gelatin, $\frac{1}{4}$ per cent. asparagin and $\frac{1}{2}$ per cent. saccharose made from decoctions of pea straw, etc. Colonies on such media fairly large, dull white, round or with irregular edges. Under the microscope smooth and homogeneous.

No liquefaction.

This species produces the peculiar root nodules on leguminous plants. At first the organisms appear as rods, later peculiar involution forms known as bacteroids develop. These bacteroids are knobbed, irregular, often forked or arranged in 3-armed masses. After these die they serve as reserve reservoirs of protein for the plants. This symbiosis between plant and bacteria brings about a considerable assimilation of atmospheric nitrogen. There may be many different species of bacteria which have this property of causing root tubercles or several varieties of one species. Commercial cultures of these bacteria are known in Germany as Nitragin.

Frank[2] believes that the nodule bacteria appear only as "swarmers," 0.9 to 1.3 $\mu$ long, which are oval or rod-like and unite in zoöglœa later. They liquefy gelatin slowly. All species are alike.

1. Beijerinck: Botanische Zeitung, 1888, p. 725.
2. Frank: Ueber die Pilzsymbiose der Leguminosen. Berlin, 1890.

### TRIBE CELLULOMONADEÆ Bergey

Short rods, occurring in soil, having the property of digesting cellulose. Motile or non-motile. Chromogenic or non-chromogenic. Growth on ordinary culture-media often not vigorous. Gram-negative.

### GENUS CELLULOMONAS Bergey

Small rods, with rounded ends, non-spore-forming, motile or non-motile, occurring in soil and having the property of digesting cellulose.

Type species *Cellulomonas biazoteus* Kellerman.

#### BACILLUS BIAZOTEUS Kellerman, McBeth, Scales, and Smith

Isolated by Kellerman, McBeth, Scales, and Smith from soil in Utah.

**Morphology.**—Rods 0.5 by 0.8 $\mu$ in dimension with 1 to 3 flagella. No endospores. Stains readily with anilin dyes. Gram-negative.

**Cultural Characters.**—*Beef Agar Slant.*—After five days abundant, raised, glistening, moist, smooth, whitish to lemon-yellow growth.

*Potato Agar.*—No growth.

*Peptone Starch Agar.*—Scant. flat, glistening, colorless growth.

*Potato Cylinders.*—In ten days a scant, flat, glistening, lemon-yellow growth.

*Gelatin Stab.*—In ten days a crateriform liquefaction 6 to 8 mm. in diameter.

*Beef Broth.*—Clouded in five days.

*Litmus Milk.*—Acid in two days. No curdling or digestion in twenty-four days.

*Cellulose Agar.*—Irregularly round colonies, small (1 to 2 mm.), opaque, gray white, with a small white nucleus. Enzymic zone $\frac{1}{4}$ mm. or less.

*Peptone Cellulose Agar.*—Round, medium sized colonies (3 to 6 mm.), semi-opaque, grayish yellow with a lemon-yellow nucleus. Usually no enzymic zone, destruction of the cellulose occurring only within the colony.

*Peptone Starch Agar.*—Round, small (0.5 to 2.5 mm.), opaque, yellowish-gray colonies with an indefinite enzymic zone, 0.5 to 3 mm. in width.

*Beef Agar.*—Round or irregularly round, small (0.5 to 2.5 mm.), translucent, grayish-yellow colonies.

**Biochemical Features.**—*Dunham's Solution.*—No ammonia in ten days. Nitrite produced.

*Starch Nitrate Solution.*—No ammonia in ten days. Nitrite produced.

*Peptone Nitrite Solution.*—No indol in ten days.

Fermentation of broth containing carbohydrates. No gas produced; percentage of acidity (Fuller's scale), with dextrose 2.0, with lactose 1.1, with saccharose 1.0, with maltose 0.9, with glycerin 0.5, with mannite 0.0, with starch 1.5.

Kellerman, McBeth, Scales, and Smith: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1913–14 xxxix, 502.

## FAMILY II. PSEUDOMONADACEÆ, Committee

Rod-shaped, short, usually motile by means of polar flagella or rarely non-motile. Aërobic and facultative. Frequently gelatin liquefiers and active ammonifiers. No endospores. Gram stain variable, though usually negative. Fermentation of carbohydrates as a rule not active. Frequently produce a water-soluble pigment which diffuses through the medium as green, blue, purple, brown, etc. In some cases a non-diffusible yellow pigment is formed. Many yellow species are plant parasites.

### GENUS 1. PSEUDOMONAS Migula, emended by Committee

Characters those of family.

A. *Producing green pigment*

Liquefying

Type species *Pseudomonas aëruginosa* (Schröter) Frost.
" *fluorescens* (Flügge) Migula.
" *Schuylkilliensis* (Wright) Chester.

Non-liquefying

*Pseudomonas Eisenbergii* Migula.
" *longa* (Zimmermann) Migula.
" *tenuis* (Zimmermann) Migula.
" *virescens* (Frick) Migula.

B. *Producing yellow pigment*

Liquefying

*Pseudomonas annulata* (Wright) Chester.
" *geniculata* (Wright).

Non-liquefying

*Pseudomonas delabens* (Wright).

C. *Producing brown pigment*

*Pseudomonas cohærens* (Wright).

D. *Non-pigmented.*

Liquefying

*Pseudomonas nebulosa* (Wright).
" *centrigugans* (Wright) Chester.
" *protea* Frost.

Non-liquefying

*Pseudomonas sinuosa* (Wright).

### PSEUDOMONAS AËRUGINOSA (Schröter) Frost

First described as *Bacillus pyocyaneus* and cultivated from blue pus by Gessard.[1]

**Morphology.**—Long thin rods, sometimes curved, measuring 0.25 by 3 to 4 $\mu$, from twenty-four-hour agar cultures when stained by gentian-violet. In many instances they are longer, measuring 0.25 by 4 to 8 $\mu$, and often grow out into long threads, 15 to 20 $\mu$. On dextrose agar twenty-four hours old the organisms are somewhat thicker, measuring 0.3 by 1.5 to 7 to 8 $\mu$ and also grow out into long threads. On both plain and dextrose agar the organisms have clinging to them a good deal of débris, peculiar knobs and protrusions. If the preparations are washed briefly in running water the organisms then appear regular and homogeneous.

**Staining Reactions.**—*Bacillus pyocyaneus* stains well by the ordinary anilin dyes, gentian-violet, carbolfuchsin, and methylene-blue. Gram-negative.

Fig. 25.—*Bacillus pyocyaneus*, plain agar twenty hours, stained with gentian-violet.

*Motility* very active, almost as rapid as that of the cholera vibrio. The organism has a single terminal flagellum.

Cultivated easily on the usual laboratory media.

**Cultural Characters.**—*Plain Agar Plates.*—In twenty-four hours the colonies are about 1 mm. in size and spreading. In forty-eight hours they are increased to 2 to 3 mm. and bottom colonies are widely spread, 1½ cm. The deep colonies are pin-point. Under low power the surface colonies are thin, granular, darker in the center, edges thin, irregular, entire. The colonies show linear markings. The deep colonies are ovals and disks. The agar shows a green discoloration about the colony. The colonies increase in size, and at the end of nine days are 6 to 8 mm. thin, with spreading peripheries. The bottom colonies are larger, 1 to 2 cm. thin and spreading. About this time the colonies reach their maximum and no further changes occur.

*Plain Agar Slant.*—Very abundant, moist, whitish growth, spreading slightly in twenty-four hours, the agar being greenish at the bottom. In three days the growth is more abundant, the agar is greenish below and a rather delicate rose-pink above, just below the growth, which has a metallic lustre. In seven days the growth is more abundant, the agar turned to deeper green, but still pinkish above. The culture retains its metallic lustre. In fourteen days the agar has practically lost its green color and has become quite pink. This remains the same for a long period.

*Plain Agar Stab.*—Abundant line and surface growth in twenty-four hours. Increased in three days along the line with a very abundant spreading surface growth. The agar is slightly pink. In seven days the growth spreads to the edge of the tube and has a metallic lustre. The agar is green just below the surface. In fourteen days growth still to the edge of the tube with a metallic lustre and green below. In twenty-one days still a metallic lustre, growth to wall of tube and agar green below.

*Dextrose Litmus Agar Plates.*—In twenty-four hours the surface colonies are 2 to 3 mm. wide, margins thin. Under low power striated, opaque, margins thin, but entire. Deep colonies pin-point. Under low power ovals and disks. Bottom colonies thin and transparent. In forty-eight hours the colonies still have traces of acid about them, but the central portion of the plates is already alkaline and blue. The colonies are larger, 5 to 6 mm. dense in the center, margins thin. They show acid centers, *i. e.*, red centers; the margins of the colonies are irregular. Under low power the margins are crenated, but entire. In four days the colonies are 8 to 10 mm. nucleated with irregular margins. The plates are still acid in some areas. Other colonies are surrounded by alkaline agar. Under low power the colonies are irregularly striped, margins entire. The agar is slightly acid. In seven days all the plates are alkaline, the colonies are larger, up to 1½ cm. in size, thin, with irregular margins. In fourteen days all the plates are alkaline. Isolated colonies may become very large, 3 to 4 cm. in diameter.

*Dextrose Litmus Agar Slant.*—In twenty-four hours very abundant, moist growth, slightly brownish, with considerable acidity. In three days the growth is thicker and more abundant, slightly pink on the surface, yellowish below. The agar is decolorized below, and is blue or alkaline above. The culture has a metallic lustre. In seven days the agar is deep blue above, the metallic lustre is more abundant. In fourteen days the entire agar is turned deep blue and in twenty-one days a dark blue.

*Gelatin Plates.*—Colonies develop slowly, appearing in forty-eight hours as hazy irregular masses, darker in the center, margins thin. Under low power they show tangled skein-like centers, margins thin, irregular and fringed. Liquefaction sets in early and in three to four days thickly seeded plates are entirely liquefied. When the colonies are not so abundant they are 3 to 4 mm. wide, lying in zones of cup-shaped liquefaction 6 to 8 mm. in diameter. The liquefied gelatin turns first green then rose-red. Isolated colonies may become 1 cm. in size. The gelatin is usually completely liquefied in nine to ten days. the liquid gelatin being either green or rose-red.

*Gelatin Stab.*—In twenty-four hours growth along line of inoculation with a beginning liquefaction on the surface. In two days an abundant line growth with the surface cupped. In seven days ½ inch surface liquefaction and tubular liquefaction along line of inoculation. Sediment in the liquid gelatin. In fourteen days almost completely liquefied. In twenty-one days completely liquefied.

*Broth.*—In twenty-four hours abundant turbidity, delicate lace-like scum, considerable granular sediment. Bright green color in broth. In three days turbidity and sediment increased and scum formation more marked. Green color is also more pronounced. In seven days abundant scum, turbidity and sediment; broth bright green. In fourteen days scum, white sediment, broth brownish. In twenty-one days the broth is purple-red, with considerable scum and sediment.

*Peptone.*—In twenty-four hours turbidity and sediment, delicate, filmy scum. Peptone somewhat muddy in color. In three days turbidity, sediment, and scum. Peptone bright green. In seven days peptone is a beautiful rose-red color, turbidity and sediment is increased. The sediment is a kind of muddy red. In twenty-one days the peptone is purple, the scum is precipitated and there is abundant sediment.

*Litmus Milk.*—In twenty-four hours no acid, milk clearing above. In forty-eight hours the digestion is more marked and in three days the milk is partially digested to a muddy yellow. In seven days digestion more advanced, the serum being yellow below and red above with the formation of a scum. In seventeen days scum is more abundant and the milk is almost completely digested to a muddy yellow. In twenty-one days digestion is complete and the serum is greenish.

*Potato.*—In twenty-four hours abundant yellow-brown growth on the surface; about the bottom of the growth a green discoloration. In three days very abundant yellow-black growth with areas of brown. In seven days covered with a black growth which is more abundant in fourteen days, and in twenty-one days the growth is abundant, blackish-brown.

*Blood-serum.*—Very abundant, moist white growth in twenty-four hours, sinking somewhat into the depths of the medium. In three days a dense, white growth somewhat sunken, with a very faint pink color at the bottom. In seven days growth is more abundant, serum softened. In fourteen days the growth has a metallic lustre with a scum in the water of condensation. In twenty-one days a partial liquefaction. The condensation of water is pink and covered with a scum.

*Fermentation Tubes.—Dextrose.*—In twenty-four hours dense turbidity in bowl, heavy scum, some sediment, arm clear. In three days turbidity and sediment in bowl, arm clear, considerable scum; no acid.

*Saccharose.*—In twenty-four hours dense turbidity in bowl, lace-like scum, broth turned greenish in the bowl. In three days turbidity in bowl increased, fragile scum, some sediment. Broth deep green; no acid.

*Lactose.*—In twenty-four hours turbidity in bowl and dense scum. Arm may be very slightly turbid; very faint sediment. In three days turbidity and scum in bowl with sediment; faint turbidity in arm; no acid.

**Pigments.**—In cultures *Bacillus pyocyaneus* produces a great variety of colors, bright green, blue-green, and reddish-brown. Gessard and Babes[2] claim that it produces 3 pigments; pyocyanin, a blue-green pigment soluble in chloroform, fluorescin, a greenish fluorescent pigment insoluble in chloroform and alcohol and soluble in water, and a reddish-brown pigment which is not characteristic. Pyocyanin is produced only by *Bacillus pyocyaneus*, while the fluorescent green pigment is found also in other organisms, especially the fluorescent water bacteria like *Bacillus fluorescens liquefaciens*. According to Boland[3] and Jordan[4] the third pigment, called pyoxanthose by Boland, is a degeneration product of pyocyanin.

Gessard has shown that pyocyanin is produced best in certain media such as neutral or slightly alkaline media containing saliva, peptone solution with or without 5 per cent. glycerin. By cultivating the organism in egg-albumin the pyocyanin production can be hindered and only fluorescin appears. Milk agar and glycerin agar favor pyocyanin production.

Certain strains of *Bacillus pyocyaneus* lose their capacity to form pyocyanin and then are difficult to distinguish from *Bacillus fluorescens liquefaciens*. The latter organism is a denitrifier however, and moreover cannot be converted into *Bacillus pyocyaneus*.

It has recently been pointed out by Meader, Robinson, and Leonard[5] that all their strains of *Bacillus pyocyaneus* produce three water-soluble pigments; the fluorescent pigment which is yellow by transmitted light and green by reflected light; pyocyanin which is bright blue; and pyorubin, which is bright red. Both pyocyanin and pyorubin are characteristic for *Bacillus pyocyaneus*.

**Ferments.**—*Bacillus pyocyaneus* produces a number of proteolytic ferments acting on gelatin, casein, fibrin, coagulated serum, and egg-albumin.

Breymann[6] believes that the gelatin-liquefying ferment is present both in the bodies of the organisms and in filtrates from broth cultures. The solution of casein may be seen in milk cultures and also on Eijkman's milk agar plates. The casein-dissolving ferment acts in alkaline solutions as trypsin does and is destroyed at 60° C.

The breaking down of protein materials does not go as far as peptone production, the solutions still clotting, and is apparently a hydrolytic cleavage.

A lipolytic ferment has been described by Neuberg and Reicher,[7] an invertin and a catalase decomposing hydrogen peroxid by Emmerich and Loew.[8] A lab-ferment has been observed by Breymann, but this apparently is not always present.

Under chloroform agar cultures of *Bacillus pyocyaneus* autolyze rapidly. This does not occur after heating to 60° to 100° C.

**Pyocyanolysin.**—The hemotoxin of *Bacillus pyocyaneus*, pyocyanolysin, was first demonstrated by Bullock and Hunter.[9] Broth cultures three to four weeks old dissolve rapidly the red blood-corpuscles of a number of animals.

Pyocyanolysin is found in the bodies of the bacilli and in filtrates from old cultures. It is destroyed at 100° C. in fifteen minutes. Its nature is not entirely clear. Antihematoxins cannot be produced against it in animals (Wassermann[10]), and Jordan regards the blood-laking property of cultures as due to alkali. It is not identical with the toxin of the bacillus. Landsteiner and Raubitschek[11] state that fresh pyocyaneus cultures on agar can be taken up in salt solution, extracted with alcohol, and leave behind on evaporation a substance which has blood-laking properties. Fukuhara[12] has obtained heat-resistant hemolytic substances from this organism by autolysis with alcohol. Ghéorghiewski[13] finds that pyocyaneus cultures also dissolve leukocytes.

**Pyocyanase.**—By killing old broth cultures of *Bacillus pyocyaneus* with chloroform, filtering, and concentrating "in vacuo," Emmerich and Loew obtained a fluid which exhibits many of the activities of fluid cultures of the organism. They found in this fluid a lab-ferment, a trypsin-like ferment, a catalase, a casease, and an invertase. In addition they found a bacteriolytic enzyme, which they called pyocyanase. Later this name was applied to the whole product. Pyocyanase liquefies gelatin, dissolves fibrin, casein, coagulated serum, and egg-albumin. It dissolves the anthrax bacillus in the test-tube and in the animal body. It also destroys other organisms like the diphtheria bacillus, staphylococci, streptococci, pneumococci, gonococci, the cholera vibrio, and the dysentery bacillus. The chemical composition of pyocyanase is not known. It is thought to be a lipoid.

**Pathogenic Action.**—*Bacillus pyocyaneus* is virulent for the usual laboratory animals, mice, guinea-pigs, rabbits, goats, pigeons, small birds, and frogs. Wassermann regards guinea-pigs as the most sensitive, especially with intraperitoneal inoculation, rabbits less sensitive, then mice and pigeons. Goats are very susceptible, dying sometimes from one oesa of a virulent culture introduced into the veins. Guinea-pigs die acutely. in one to three days from intraperitoneal inoculation. At autopsy they show an acute peritonitis, with a

flocculent often hemorrhagic exudate, and areas of bronchopneumonia. With subcutaneous inoculation they live longer, developing an acute edema at the site of inoculation which may subside or lead to a necrosis and slough. In rabbits intraperitoneal inoculation produces a hemorrhagic peritonitis, intravenous injection a septicemia. Brau[16] has infected rabbits by feeding.

**Antagonism to Other Bacteria.**—The injection of virulent pyocyaneus cultures into animals infected with *Bacillus anthracis* has a favorable influence on the course of the infection as first shown by Bouchard.[15] It was subsequently pointed out by Blagovestchensky[16] that when *Bacillus pyocyaneus* and *Bacillus anthracis* are streaked cross-wise on agar plates there is a definite inhibition of the growth of the anthrax where the streaks cross. There is a similar antagonism between *Bacillus pyocyaneus* and staphylococci and streptococci (Trombetta[17]), and gonococcus (Grosz and Kraus[18]).

**Pyocyaneus Toxin and Antitoxin.**—Filtered cultures of *Bacillus pyocyaneus* are toxic to rabbits and guinea-pigs as first shown by Charrin.[19] This toxic action was further investigated by Wassermann, who found that in old broth cultures killed by toluol and kept in the incubator for long periods (forty days) a highly toxic fluid could be obtained. There was a great difference in strength depending upon the strain used. Good toxins were acutely poisonous to guinea-pigs, pigeons, and mice by small doses, 0.2 to 0.25 c.c. given intraperitoneally. This toxic body was heat-resistant, not being destroyed at 100° C. in five minutes. It is regarded by Wassermann as a true extracellular toxin. Small animals like guinea-pigs and rabbits may be actively immunized with both living organisms and with toxins, a definite resistance being established as early as seven days. By the use of living cultures Wassermann obtained in goats a bactericidal serum, by the use of dead cultures and filtrates an antitoxic serum. The antitoxic serum neutralizes pyocyaneus toxin; 0.3 to 0.5 c.c. neutralizing a quantity equal to ten times a fatal dose for guinea-pigs. It was not possible to neutralize larger quantities, possibly because *Bacillus pyocyaneus* has an endotoxin as well as an exotoxin.

By the immunization of rabbits with living organisms agglutinins are produced, acting in a dilution of 1 : 1000, and precipitins as well.

**Action of Bacillus Pyocyaneus in Man.**—*Bacillus pyocyaneus* is the etiological agent in a variety of pathological conditions in man of which the best known is that of "blue or green pus." It was first recognized by Schimmelbusch[20] that the organism, while it can cause local inflammation and general symptoms, never invades the body, at least in adults. It lives as a saprophyte on the skin and in the intestinal tract. As shown by Kossell,[21] however, this species is very virulent for young children, being found in middle-ear disease and its complications, mastoiditis and meningitis. It is also found in abscesses of the navel in the newborn (Babes),[21] in hemorrhagic pustules in the newborn, and in the heart's blood seven hours after death (Ehlers[22]). It is not infrequently found as a cause of septicemia in young infants, being present in the blood and in the organs which show definite tissue reactions. This condition is known as "la maladie pyocyanique."

In mixed infections in wounds the pigment production of *Bacillus pyocyaneus* is much influenced, being lost with *Staphylococcus aureus* and *Bacillus anthracis* (Schimmelbusch and Mühsan[23]), with the streptococcus (Krause[24]).


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   ———: Ann. de l'Inst. Pasteur, 1890, iv, 88. *Ibid*, 1919, xxxiii, 241. *Ibid*, xxxiv, 88.
2. Babes: Comp. rend. Soc. de biol., 1889, i, n. s., 438.
   ———: Bakteriologische Untersuchungen über, septische Processe des Kindersalter. Leipzig, 1889.
3. Boland, Centralbl. f. Bakeriol [etc.], 1899, xxv, 897.
4. Jordan: J. Exper. Med., 1899, iv, 627.
   ———: J. Med. Research, 1903–04, x (n. s. v), 31.
5. Meader, Robinson, and Leonard: Am. J. Hyg., 1925, v, 682.
6. Breyman: Centralbl. f. Bakteriol. [etc.), 1902, xxxi, 481.
7. Neuberg and Reicher: München. med. Wchnschr., 1907, liv, 1725.
8. Emmerich and Loew: Ztschr. f. Hyg., 1901, xxvi, 9.
9. Bullock and Hunter: Centralbl. f. Bakteriol. [etc.], 1900, xxviii, 865.
10. Wassermann: Ztschr. f. Hyg., 1896, xxii, Bacillus pyocyaneus.
    *In*: Handb. d. Path., Mikroorg. (Kolle and Wassermann). 1. Aufl., 1903, iii, 471.
11. Landsteiner and Raubitschek: Centralbl. f. Bakteriol. [etc.], 1908, xlv, 660.
12. Fukuhara, Arch. f. Hyg., 1909, lxxi, 387.
    ———: Ztschr. f. Immunitätsforsch., 1911, ix, 77.
13. Ghéorghiewski: Ann. de l'Inst. Pasteur, 1899, xiii, 298.
14. Brau: Compt. rend. Soc. de biol., 1906, lxi, 275.
15. Bouchard: Compt. rend. Soc. de biol., 1889, i, 714.
16. Blagovestchensky: Ann. de l'Inst. Pasteur, 1890, iv, 689.
17. Trombetta: Centralbl. f. Bakteriol. [etc.], 1892, xii, 122.
18. Gross and Kraus: Arch. f. Dermat. u. Syph., 1898, xlv, 329.

19. Charrin: La malaide pyocyanique. 1889.
———: [Abstr.] Centralbl. Bakteriol. [etc.], 1897, xxii, 58.
20. Schimmelbusch: Volkmann's klin. Vortr., 1893, No. 63.
21. Kossell: Ztschr. f. Hyg., 1898, xvi, 368.
22. Ehlers: Hospital Tidende Kopenhagen. 1890.
23. Schimmelbusch and Mühsam: Arch. f. klin. Chir., 1893, xlvi, No. 4.
24. Krause: Centralbl. f. Bakteriol. [etc.], 1900, xxvii, 769.

### PSEUDOMONAS FLUORESCENS (Flügge) Migula

Described originally by Flügge[1] as *Bacillus fluorescens liquefaciens*. Named by Migula.[2] Common in water and in putrefying infusions. Present description from a culture obtained from the American Museum.

**Morphology.**—Rather stumpy bacilli on plain agar in twenty-four hours, not so slender as *Bacillus pyocyaneus* and ends not so square. The organisms measure about 0.5 $\mu$ in width and 1.5 to 2.5 $\mu$ in length. On dextrose agar in twenty-four hours they show a definite en-

Fig. 26.—*Bacillus fluorescens liquefaciens*, plain agar twenty hours, stained with gentian-violet.

largement, measuring 0.5 to 0.6 $\mu$ in width and 2.5 to 3 $\mu$ in length. The ends are somewhat pointed and whip forms are present, measuring 0.5 $\mu$ in width and 7 to 10 $\mu$ in length.

**Staining Reactions.**—Stained easily by the ordinary anilin dyes. Gram-negative.

**Motility.**—Active in young cultures. In old cultures the organisms may become non-motile. Single polar flagella.

**Plain Agar Plates.**—In twenty-four hours the colonies are small, delicate, flat, $\frac{1}{2}$ to $\frac{3}{4}$ mm. in diameter. Under low power they are flat, opaque, slightly nucleated; margins slightly dentated. Deep colonies pin-point, under low power pale, irregular with entire margins. In three days the colonies are slightly increased in size, and in six days the colonies are much larger, measuring 3 to 4 mm., delicate and flat. The bottom colonies spread widely, have a bluish color, and are 1 cm. in size. The deep colonies are now distinct ovals and disks. The agar is slightly greenish. In fifteen days the surface colonies are slightly increased in size, but still delicate, varying from 4 to 5 mm. in diameter. Under low power the margins are thin and the surface fringed.

*Plain Agar Skant.*—In twenty-four hours delicate, filmy, rather spreading growth. The agar is slightly greenish. Growth increased in forty-eight hours, and in three days a delicate,

flat growth occurs, spreading slightly at the edges. In two weeks the growth is thin and spreading with a very slight greenish color in the agar.

*Plain Agar Stab.*—In twenty-four hours delicate line and surface growth. In three days more abundant, especially on the surface. Agar just below the surface greenish. In two weeks moderate line growth, abundant surface growth, somewhat pinkish in color.

*Dextrose Agar Plates.*—Surface colonies in twenty-four hours, $\frac{1}{2}$ to $\frac{3}{4}$ mm. in size, thin, bluish. Under low power they are granular with nucleated margins, thin, irregular, and striated. Deep colonies pin-point, under low power round and oval with irregular crenated margins. No acid in twenty-four hours, but in forty-eight hours the plates are acid in part and the colonies larger somewhat, measuring about 2 mm. In five days the plates have become alkaline and the colonies measure 5 to 6 mm., are nucleated, with thin margins. On plate with few colonies some of the colonies are still acid and others are alkaline. In seven days the colonies are practically all alkaline and slightly greenish. They are darker in the center with thin margins. In eighteen days the plates are all alkaline and the agar slightly greenish-blue. Old colonies are pale, flat, 1 cm. in size, with irregular margins. Deep colonies small, delicate, oval, almost pin-point in size, measuring about $\frac{1}{4}$ mm.

*Dextrose Agar Slant.*—In twenty-four hours abundant, thick, white growth, with slight acidity. Growth increased in forty-eight hours, and in three days a thick, abundant somewhat greenish-white growth develops. Acidity lessened. In two weeks a thin, whitish, moist, spreading growth occurs and the agar is highly alkaline.

*Broth.*—In twenty-four hours turbidity and sediment with scum; the broth bright green. In three days turbidity, sediment, and scum are increased. The broth is slightly more greenish. In two weeks heavy turbidity, delicate scum, some sediment. Broth bright green.

*Petpone.*—In twenty-four hours turbidity, sediment, and scum, with a faint greenish color. In three days turbidity, sediment, and scum are increased. Color now definitely green. In two weeks faint turbidity, some sediment, scum precipitated. Peptone muddy green.

*Litmus Milk.*—No change in reaction. The milk proteins are gradually digested, clearing from above downward. In two weeks digestion is complete, a brownish fluid resulting.

*Potato.*—In twenty-four hours faint, brownish-yellow growth. More abundant in three days, flat, dry, and greenish-brown. Still more abundant in two weeks.

*Blood-serum.*—In twenty-four hours delicate, brownish growth. Water of condensation green. In three days abundant, moist, whitish growth. In two weeks smooth, moist growth, some softening of the serum. In twenty-one days considerable softening of the serum. In older cultures the serum is softened, but not definitely liquefied.

*Gelatin Stab.*—Growth begins in forty-eight hours, abundant along line of inoculation and spreading on the surface. In three days the line growth is more distinct and there is a surface liquefaction involving one-fourth of the tube. The liquid gelatin is greenish and in six days the tube is one-half liquefied. In ten days the gelatin is completely liquefied and is greenish in color.

*Gelatin Plates.*—Colonies become visible in forty-eight hours, small, delicate, hazy, $\frac{1}{2}$ mm. Under low power they are faintly striated, edges jagged or irregular, margins thin. Contents granular. In three days the colonies are larger, $\frac{1}{2}$ to $\frac{3}{4}$ mm. in size, each lying in a cup of liquid gelatin, varying from 4 to 8 mm. in diameter. In five days the plates are entirely liquefied. The gelatin does not develop a greenish color.

**Fermentation Tubes.**—*Dextrose.*—In twenty-four hours turbidity in bowl, filmy scum, arm clear, no pigmentation. In three days lacy scum, turbidity and sediment increased, arm clear. Possibly faint acid reaction. No color.

*Saccharose.*—In twenty-four hours turbidity, sediment, and scum. Arm clear. Broth bright green. In three days turbidity, heavy scum, and sediment. Arm clear, broth bright green. No acid.

*Lactose.*—In twenty-four hours dense turbidity, filmy scum, and sediment. Arm clear. Broth faint green in color. In three days turbidity, lacy scum, and sediment increased. Arm clear. Slightly greenish color in bowl. No acid.

1. Flügge: Die Mikroorganismen. 2. Aufl., 1886, ii, 289.
2. Migula: p. 886.

### PSEUDOMONAS SCHUYLKILLIENSIS (Wright) Chester

Described by Wright[1] from the Schuylkill River as *Bacillus fluorescens Schuylkilliensis.* Named by Chester.[2]

**Morphology.**—A rather small, short, motile bacillus, with rounded ends, occurring often in pairs and sometimes in threads and long forms. It has a polar flagellum. No spore-formation observed.

**Culture.**—*Colonies in Gelatin.*—On the second day the surface colonies appear as grayish-white, semitranslucent disks, with smooth, sharply defined outlines, and a diameter of

about 1½ mm. Under a low magnifying power the colony is seen to be brownish and granular toward the center, but thin and translucent toward the periphery. The outline is smooth or slightly wavy, and sharply defined. From the center of the colony brownish, indistinct, wavy, broken lines are seen to radiate toward the margin. The deep colonies are rounded or oval, dark brownish and granular, with sharply defined outlines. In these, also, faint brownish lines are seen radiating from the center. Later the surface colonies get thicker and larger and may have a greenish-white color, while the surrounding gelatin acquires a bluish-green fluorescence. The colonies gradually sink into the gelatin and a slow liquefaction begins, the colony losing its sharply defined outlines and being surrounded by a narrow zone of cloudy liquefied gelatin.

*Gelatin Stab.*—Slow liquefaction; first an excavation in deep saucer form, several millimeters in diameter and lined by a grayish-white, thick layer, which is fairly smooth and shining. This excavation increases somewhat in size and liquefaction slowly occurs beneath the lining membrane, while the non-liquefied gelatin near the surface acquires a marked bluish-green fluorescence. There is only a faint growth along the line of inoculation.

*Acid Gelatin.*—Good growth; less fluorescent.

*Agar Slant.*—Grayish, semitranslucent stripe, with sharply defined, wavy margins; the agar becomes fluorescent.

*Bouillon.*—Clouded; on the surface a delicate grayish pellicle forms, while the bouillon is bluish-green fluorescent; there is a stringy sediment.

*Potato.*—Chocolate-colored, viscid-looking, rather thick, and rather widely spreading growth.

*Litmus Milk.*—Slow coagulation and cloudy serum containing flocculi in suspension; the clot is jelly-like and decolorized, and is slowly dissolved; there is a pink ring on tube wall above a pale bluish ring; the reaction is alkaline.

*Sugar gelatin* in deep stab. Very faint development.

*Rosolic Acid.*—Color is deepened.

*Indol Production.*—The reaction is doubtful or faint; the addition of nitrite is necessary.

*Relation to Temperature.*—Does not grow at 35° to 36° C.

1. Wright: Memoirs Nat. Acad. Sc., 1894, vii, 422–474.
2. Chester: Manual of Determinative Bacteriology. New York, 1914, p. 320.

### PSEUDOMONAS EISENBERGII Migula

Noted in water by Eisenberg[1] as *Bacillus fluorescens non-liquefaciens.* Named by Migula.[2] Probably the form described by Lustig[3] as *Bacillus aquatilis fluorescens* is the same species.

**Morphology.**—Short fine bacilli with rounded ends. Non-motile. No spores.

*Grows* rapidly, only under aërobic conditions, and at low temperatures (25° C.).

**Cultural Characters.**—*Agar Slant.*—Surface expansion producing a green color in the agar.

*Gelatin Plates.*—Surface colonies leaf-like with mother-of-pearl opalescence. No liquefaction.

*Gelatin Stab.*—Slight growth along line of inoculation with a greenish fluorescent surface expansion. No liquefaction.

*Potato.*—Rapid growth, at first brownish, then bluish-gray in color. Pigment greenish-yellow, fluorescent.

Bergey[4] reports this species as Gram-negative, not forming indol or reducing nitrates. It produces a turbidity and fluorescence in broth and does not change litmus milk.

1. Eisenberg: Bakteriologische Diagnostik. 1891, p. 145.
2. Migula: p. 913.
3. Lustig: Diagnostik der Bakterien des Wassers. p. 64.
4. Bergey: p. 132.

### PSEUDOMONAS LONGA (Zimmermann) Migula

Found by Zimmermann[1] in the Chemnitz water supply and described as *Bacillus fluorescens longus.* Named by Migula.[2]

**Morphology.**—Bacilli varying greatly in size and measuring 0.83 $\mu$ in breadth and from 1.45 to 1.65 $\mu$ in length, with long forms 14 $\mu$ or more in length. The individual organisms are straight or slightly curved, with rounded ends.

*Motility.*—The short rods are actively motile. The long threads show slow vibratory or serpentine movements.

*Staining Reactions.*—The organisms stain well with the ordinary anilin dyes. Gram-positive. The individual rods show well-marked polar bodies. The flagella may be demonstrated by Loeffler's stain if a small amount of sodium hydrate (4 drops) is added to the mordant. They are very fine, slightly wavy, or bent in half circles, 1 to 5 in number, usually attached to one end of the rod, rarely appearing on both ends. They vary in length, being usually longer than the shorter rods, but not so long as the long forms.

*Rapid growth* at room temperature, only under aërobic conditions.

**Cultural Characters.**—*Agar Slant.*—Moderately thick surface expansion, the agar turning greenish-yellow.

*Gelatin Plates.*—Colonies appear in twenty-four hours as small whitish points which grow rapidly and in four days are 1 cm. in diameter. The deep colonies are light grayish-green in color. The surface colonies appear first as delicate transparent almost circular flecks with a mother-of-pearl iridescence. Later they are more opaque and take on a dirty yellow-green color. The edges are convoluted and not clearly differentiated from the gelatin. Under low power the deep colonies are dark and brownish, coarsely granular, show many parallel linear markings, and have irregular edges. The surface colonies under low power are light brown in color, round, convoluted, with lighter edges, which gradually thin out and are only slightly raised from the gelatin. Later the convolutions become more marked and somewhat resemble the intestines of a small animal. Old colonies show dark yellowish-green centers surrounded by almost colorless transparent edges in which fine radial folds are visible. Under low power the deep colonies are now very dark, egg-shaped, with sharp edges. The surface is smooth, the striations practically lost. On old plates the colonies are very irregular and reveal a characteristic whirl of lines and indentations. The gelatin is not liquefied.

*Gelatin Stab.*—Surface growth very thin, with a heaped-up mass in the center. The growth quickly reaches the edge of the gelatin, which assumes first a bluish, then a bluish-green fluorescence.

*Gelatin Streak.*—Fern-like expansion from the line of inoculation which seems to represent the stalk.

*Potato.*—Moist thin expansion, grayish-yellow in color, covering the entire surface.

*Broth.*—Turbidity and flocculent masses of bacteria which gradually settle to the bottom and form a thick grayish-white sediment. A thin film develops on the surface, while the fluid becomes deep yellowish-green in color.

1. Zimmermann: Die Bakterien unserer Trink-und Nützwässer. 1. Reihe, 1890, p. 20.
2. Migula: p. 907.

### PSEUDOMONAS TENUIS (Zimmermann) Migula

Described originally as *Bacillus fluorescens tenuis* by Zimmermann[1] from the Chemnitz water. Named by Migula.[2]

**Morphology.**—Short thick bacilli measuring 0.8 $\mu$ in thickness and 1 to 1.8 $\mu$ in length in stained preparations (Zimmermann). According to Migula the living organisms vary in length from 1.4 to 3 $\mu$ and are usually about 2 $\mu$ long. In young cultures the organisms are single or in pairs, while in old cultures they form short threads which adhere in flocculent packets. Ends roundish or pointed. In old cultures the organisms show spore-like enlargements at the poles, sometimes at one, sometimes at both ends. True spores not formed.

*Motility* oscillating and rotatory.

*Stains* with difficulty, best with alkaline aqueous anilin fuchsin after a mordant of 1 per cent. tannin. Gram-negative. The flagella are difficult to demonstrate because of the viscid material in which the organisms are embedded. They are best stained by using a mordant with considerable sodium hydrate added (9 drops). They arise as bunches from one or both poles, are about three times as long as the organisms, and are provided with regular waves. In old cultures the organisms show 3 to 5 cilia at each pole, while in actively growing organisms the bunches are much thicker, often containing as many as 25. Here they look like "Medusa heads" and seem to arise from the sides of the rods.

*Growth* rapid, best at room temperature, only under aërobic conditions.

**Cultural Characters.**—*Agar Slant.*—Gray, flat, not very abundant, glistening expansion. The agar assumes a greenish color.

*Gelatin Plates.*—Fairly large, thin iridescent colonies on the surface in two days, grayish and transparent, edges radially striated. At the end of three days these colonies are about 6 mm. wide. Under lower power the deep colonies are light brownish-gray in color; irregularly round, oval or egg-shaped, with slightly indented edges. The contents are granular and marked with irregular lines. No liquefaction.

*Gelatin Streak.*—Thin, glistening, gray expansion with radial striations on the edge. At the end of three days this may be 6 mm. in width. The gelatin turns green even at considerable distance from the growth.

*Gelatin Stab.*—Very thin grayish-white expansion which gradually becomes slightly thicker and reaches the wall of the tube in about three days. Faint growth along the line of the stab. The gelatin turns yellow while the growth appears at first blue by reflected light, then blue-green. No liquefaction.

*Potato.*—Thin gray-yellow glistening expansion spreading slowly from the line of inoculation. Eventually the growth becomes reddish-brown.

*Broth.*—Turbidity and abundant whitish or brownish flocculent sediment. Thin film forms on the surface which on shaking falls to the bottom in a cloud-like mass. The upper third of the fluid first turns greenish-yellow, then the color extends to the bottom of the tube.

1. Zimmermann: Die Bakterien unserer Trink-und Nützwässer. 1. Reihe, 1890, p. 16.
2. Migula: p. 910.

### PSEUDOMONAS VIRESCENS (Frick) Migula

Found in green sputum by Frick.[1] Named *Bacillus virescens* by Migula.[2]

**Morphology.**—Slender bacilli slightly longer and thinner than the typhoid bacilli. The length is 5 to 6 times the breadth in living organisms, 4 to 5 times in organisms mounted in balsam. Ends somewhat rounded. No spores.

*Motility* active, vibratory, progressive, and serpentine.

**Cultural Characters.**—*Agar Slant.*—Dry, whitish, glistening mass along entire line of inoculation, thicker in the center, somewhat flatter toward the edges. Development ceases after three to five days at room temperature. Rarely the growth extends as a flat, white expansion to the walls of the tube. Usually the growth is only 4 to 5 mm. wide. The underlying agar turns light green and after two to three days this green color becomes more intense. Later it becomes yellow-brown.

*Agar Stab.*—White, glistening, surface expansion at the point of inoculation, somewhat thin, not more than 4 to 5 mm. in diameter. No growth in the depths. Greenish color develops slowly and sparsely in the agar, later becoming yellow-brown.

*Gelatin Plates.*—Fine colonies develop in twenty-four hours, appearing under the microscope as sharply limited and circular. Deep colonies sharply contoured, darker in the center, brownish, fine, granular. Surface colonies later almost circular, sharply circumscribed with fine lines in the edges. The colonies are flat, not raised from the surface, finely granular, usually light colored. The agar takes on a bright green color. Average size of colonies forty-eight hours old about 2 to 2.5 mm. in diameter. The colonies frequently show a central button-like thickening.

*Gelatin Stab.*—Flat expansion at the surface about 3 mm. in diameter in forty-eight hours. Sparse growth in the depths, consisting of a chain of fine grayish points. The upper part of the gelatin turns yellowish-green. Later the surface growth increases in size, but remains thin and delicate grayish in color.

The gelatin takes on a dark green color extending to the wall of the tube and about 1 cm. in depth. Later the color becomes yellow-brown to brown with a greenish fluorescence.

*Gelatin Streak.*—Growth appears as a lance-shaped colony with irregular contours and gradually spreads to the walls of the tube. The expansion is delicate and finely granular. The agar is first green, then turns yellow-brown to brown, with an abundant green fluorescence.

*Broth.*—Abundant turbidity in twenty-four hours with a surface greenish fluorescence. Surface scum pearl-gray in color extending to the edge of the tube. If the scum be disturbed it sinks to the bottom and a new scum forms with another fluorescence. Eventually the entire mass of broth becomes green.

*Potato.*—Abundant growth about 1 mm. thick in forty-eight hours, brownish to nut-brown in color. Growth usually limited to line of inoculation. Potato takes on a dirty violet color.

*Milk.*—Rapid coagulation in large clumps. These gradually become smaller and finally disappear, only a yellowish watery whey being left with small flocks of casein floating about in it. The filtrate of this always shows peptone. Old milk cultures have an acid reaction from lactic acid.

1. Frick: Virchow's Arch., 1889, cxvi, 266.
2. Migula: p. 916.

### PSEUDOMONAS ANNULATA (Wright) Chester

Described as *Bacillus annulatus* by Wright.[1] Named by Chester.[2] Isolated from the water of the Schuylkill River.

**Morphology.**—A rather small motile bacillus, with rounded ends, occurring singly, in pairs, and longer forms. It has several flagella apparently situated at one or both ends.

**Cultural Reactions.**—*Colonies* in *Gelatin.*—About the third or fourth day the colonies

have formed round, saucer-shaped liquefactions, 2 or 3 mm. in diameter; there is a yellowish mass at the center, and this is surrounded by a cloudy zone of liquefied gelatin. Under the low magnifying power a thick, opaque yellowish clump is seen at the center, around this dark granular material, and at the margin of the liquefaction sometimes an indistinct fringe of short hair-like processes, or the outline may be ill defined; motion of a circulating character may be observed in the liquefied area. The deeper colonies, under the low power, are oval or irregularly round, brownish, finely granular, and sharply defined. If the colonies are few in number the liquefactions may attain a diameter of 7 mm. or more, and may show one or two concentric, hazy, yellowish rings formed of yellowish flocculi.

*Gelatin Stab.*—Liquefaction is saucer or cup form, with only faint development along the line of inoculation. The liquefied gelatin is clouded, and an abundant yellow flocculent deposit is seen at the bottom; there may be a delicate, somewhat iridescent pellicle on the surface; the liquefied gelatin has an alkaline reaction.

*Acid Gelatin.*—Good growth; no growth in line of inoculation.

*Agar Slant.*—Warm, yellow, semitranslucent stripe, with wavy margins and a smooth shining surface.

*Bouillon.*—Clouded, and flocculi in suspension. A yellow pellicle forms on the surface, and there is an abundant, thick, membranous sediment of a yellow color; the reaction is alkaline.

*Potato.*—Thin, moist, spreading, brown-yellow growth.

*Litmus Milk.*—Coagulated and decolorized; clot rather firm and serum clouded; pink ring on tube wall; yellowish deposit at bottom of tube; reaction is acid.

*Rosolic Acid.*—Color much deepened; reaction is alkaline.

*Indol Production.*—Faint reaction; does not produce nitrites.

*Relation to Temperature.*—Does not grow at 35° to 36° C.

1. Wright: l. c., 422.
2. Chester: l. c., 315.

### PSEUDOMONAS GENICULATA (Wright) Chester

Reported originally by Wright[1] as *Bacillus geniculatus*. Isolated from the water of the Schuylkill River. Rare. Named by Chester.[2]

**Morphology.**—A medium-sized motile bacillus, occurring singly, in pairs, and sometimes in threads. It is a polar flagellated bacillus, the number of flagella varying from one to probably four; no spore formation observed.

**Cultural Characters.**—*Colonies in Gelatin.*—About the second day the surface colonies are rounded, semitranslucent, whitish disks, somewhat denser at the center and 1 mm. to 2 mm. in diameter. They may already be slightly depressed in gelatin; under a low power they are brownish and granular about their centers, thinner at their margins, which are smooth or slightly wavy, and sharply defined in colonies not yet beginning to liquefy the gelatin, while in those which are, the margins are rugged and surrounded by a finely granular zone, apparently made up of delicate, radiating fibrils; the colonies soon get denser as liquefaction begins, and may have a greenish shimmer. The deep colonies appear to the naked eye as yellow points; under a low magnifying power they are spherical, sharply defined, faintly granular, and are studded here and there by small plaques or buds. About the third day the colonies are circular in outline, grayish-white with yellowish centers, and several millimeters in diameter; they lie in saucer-shaped depressions containing liquefied gelatin, a narrow zone of which surrounds them; later a pinkish tint may be observed at their centers; under a low power they are now granular, dark, and dense, with a greenish shimmer, breaking up at the margin into a granular zone, which is bounded by a more or less distinct fringe of radiating fibrils. Motion of a circulatory character has been observed in the liquefied gelatin; the deep colonies, when examined with the low power about this time, are rough looking, apparently being made up of aggregations of small, irregular clumps, an appearance which seems to be the result of the budding process noted above. Many have a dark center and may show a reddish-brown tint or a greenish shimmer.

*Gelatin Stab.*—Liquefaction in funnel form extending slowly down the line of inoculation; there may be a constriction at the top inclosing an air space. The liquefied gelatin is clouded and there is an abundant whitish to pinkish deposit; there may be a thin pellicle on the surface; the growth along the line of inoculation preceding liquefaction has a pinkish tint, and may be composed of discrete and confluent spherical colonies.

*Acid Gelatin.*—Grows less vigorously and with slow liquefaction, which does not extend along the line of inoculation.

*Agar Slant.*—Grayish, shining, smooth surface growth, not spreading widely, with wavy, bulging margins; it is semitranslucent by transmitted light. The agar acquires a brownish-green tint.

*Bouillon.*—Clouded with flocculi in suspension, which at the surface may form an im-

perfect pellicle; the bouillon acquires a slight greenish tint; there is a whitish sediment at the bottom.

*Potato.*—Thin, viscid looking; moist, shining, brownish growth, not widely spreading.

*Litmus Milk.*—Coagulated and decolorized; there is a pinkish-yellow scum on some cloudy serum; the clot is jelly-like; the serum after a time may have a slightly greenish tint; there is a bluish ring on the tube wall; the reaction is alkaline.

*Sugar Gelatin in Deep Stab.*—Points of liquefaction containing pinkish flocculi along the line of inoculation; later liquefaction all along the line irregularly, and here and there pinkish clumps; in places groups of delicate lateral furry outgrowths; no gas is produced.

*Rosolic Acid.*—Color deepened.

*Indol Production.*—Negative.

*Relation to Temperature.*—Does not grow at 35° to 36° C.

1. Wright: l. c., 422.
2. Chester: l. c., 313.

### PSEUDOMONAS DELABENS (Wright) Chester

Described as *Bacillus delabens* by Wright.[1] Isolated from the water of the Schuylkill River. Common. Named by Chester.[2]

**Morphology.**—A rather small motile bacillus, with rounded ends, occurring in short, rounded and long forms and in pairs; it has a terminal flagellum; no spore-formation observed.

**Colonies.**—*Colonies in Gelatin.*—About the second day the surface colonies are thin, translucent, shining expansions, with wavy, irregular, sharply defined outlines and grayish centers. Under a low magnifying power the colony is thin and translucent, and the surface is marked by numerous delicate lines which run in various directions, and which sometimes branch. The outline is sharply defined, wavy, and irregular; toward the center a brownish-yellow color appears, and a sharply defined yellow-brown refractive central nucleus may be observed. The colony soon becomes denser and grayish-white throughout, and sinks into the gelatin, which is slowly liquefied. The deep colonies, under a low magnifying power, are rounded or slightly irregular in outline and sharply defined; they are slightly granular and of a brownish-yellow tint, often darker toward the center. A dark ring may sometimes be observed between the center and the periphery.

*Gelatin Slant.*—A smooth, grayish-white stripe with wavy margins is formed at first; this sinks into the gelatin, and a furrow is formed lined by a grayish-white, viscid layer; liquefaction slowly occurs and the growth to some extent gradually slips downward; the surface of the non-liquefied gelatin acquires a hazy appearance.

*Gelatin Stab.*—A thin, grayish-white surface expansion with irregular outlines is formed; this slowly sinks into the gelatin, which is slowly liquefied; there is a fair growth along the line of inoculation of brownish-gray, discrete, and confluent colonies.

*Acid Gelatin.*—The growth is perhaps less vigorous.

*Agar Slant.*—Whitish, semitranslucent, shining growth, not widely spreading; the agar after a time acquires a slight green tint.

*Bouillon.*—Clouded; whitish flocculi may form an imperfect pellicle on the surface; there is a whitish sediment, and the bouillon may acquire a slight green tint.

*Potato.*—Brownish, viscid, rather thick growth, spreading rather widely and becoming darker in older cultures.

*Litmus Milk.*—Decolorized; after a few weeks there is a smoky bluish layer at the top, while below this the milk has a brownish-white color; there may form a tough membrane on the surface which permits the tube to be inverted without disturbing the liquid; above this there is a bluish ring on the tube wall; there is no coagulation, but the milk may be viscid in old cultures; the reaction is alkaline.

*Sugar Gelatin in Deep Stab.*—No growth.

*Rosolic Acid.*—Rose color much deepened.

*Indol Production.*—Faint reaction; no nitrite production.

*Relation to Temperature.*—Does not grow at 35° to 36° C.

1. Wright: Mem. Nat. Acad. Sc., 1894, vii, 422.
2. Chester: l. c., 314.

### PSEUDOMONAS COHÆRENS (Wright) Chester

Described first as *Bacillus cohærens* by Wright.[1] Isolated from the water of the Schuylkill River. Seems to be a common organism. Named by Chester.[2]

**Morphology.**—A medium-sized, short, motile bacillus with rounded ends, occurring often in pairs and sometimes in long forms; it has a polar flagellum; no spore-formation observed.

**Cultural Characters.**—*Colonies in Gelatin.*—About the fourth or fifth day the surface colonies are round, elevated, grayish, semitranslucent disks, about 1 to 2 mm. in diameter, with sharply defined outlines. Under a low power they are finely granular, refracting, and slightly yellowish-brown in tint toward their centers, where there is a nucleus. The outline is smooth and rounded and sharply defined. The deep colonies under the low power are round or oval, granular, brownish, and sharply defined in outline. The surface colonies may increase somewhat in size, becoming thicker, denser, and whiter, with an elevated brownish nodule at their centers. Later they sink into the gelatin and their margins may have a "crimped" appearance. The deep colonies after some days become more brown in color, when examined under a low power, and there may be a slight brownish tint in the gelatin immediately surrounding them, which is not apparent to the naked eye. Sometimes the deep colonies may form morula-like aggregations of daughter colonies.

*Gelatin Slant.*—A more or less wrinkled, viscid looking, grayish-white membrane lining a rather deep furrow in the gelatin, which is very slowly liquefied.

*Acid Gelatin.*—Grows well.

*Agar Slant.*—An irregular, somewhat elevated, grayish-white, semitranslucent, shining growth with irregular margins.

*Bouillon.*—Clouded. A coherent, viscid-looking, tenacious, much-wrinkled membrane forms on the surface and adheres to the wall of the tube. In older cultures the bouillon seems to become quite clear.

*Potato.*—A rather thick, granular looking, widely spreading growth of the color of the potato.

*Litmus Milk.*—Decolorized and a viscid coagulation with a layer of smoky, bluish-colored serum at the top and a coherent, thick, tough membrane on the surface. The milk acquires a dirty, brownish-white color and there is a bluish ring on the tube wall. The casein seems to be slowly dissolved; the reaction is alkaline.

*Sugar Gelatin in Deep Stab.*—Fair growth of discrete colonies; no gas production.

*Rosolic Acid.*—Color deepened.

*Indol Production.*—Negative.

*Relation to Temperature.*—Grows at the temperature of the room; its growth at 35° to 36° C. is doubtful.

1. Wright: Mem. Nat. Acad. Sc., 1894, vii, 422–474.
2. Chester: l. c., 312.

### PSEUDOMONAS NEBULOSA (Wright) Chester

Noted originally by Wright[1] as *Bacillus nebulosus.* Isolated from the water of the Schuylkill River. Named by Chester.[2]

**Morphology.**—A medium-sized, motile bacillus, with rounded ends occurring singly and in pairs; in length it is somewhat variable. It has a polar flagellum; no spore-formation observed.

**Cultural Characters.**—*Colonies in Gelatin.*—On the third day the typical surface colony is a thin, gray, translucent, hazy, round, outlined expansion, with a well-marked whitish center, surrounded by a whitish ring. The diameter may be about 3 mm. Under a low power a dark brownish, granular nucleus is seen at the center, around which a dark, finely granular zone extends about halfway to the periphery and then gives way rather abruptly, by the appearance in it of discrete and confluent, sharply defined, translucent areas, to a very thin, transparent zone, which ends at the well-defined, nearly smooth margin. The deep colonies are rounded, dark, and granular, sometimes brownish-green in tint.

*Gelatin Slant.*—A viscid whitish layer lines a shallow furrow, on each side of which thin, hazy, short, lateral outgrowths on the surface of the gelatin are seen, giving the growth a feathered appearance. By transmitted light the layer in the fissure is yellowish-white. There is slow liquefaction.

*Acid Gelatin.*—Does not seem to grow.

*Agar Slant.*—A very thin, translucent stripe with irregular margins.

*Bouillon.*—Clouded and whitish sediment.

*Potato.*—Very scanty growth, if any.

*Litmus Milk.*—No definite coagulation; it is decolorized and the casein seems to be dissolved. On the surface a broken, wrinkled, whitish mycoderma. The reaction is alkaline.

*Sugar Gelatin in Deep Stab.*—Fair growth, with some gas formation.

*Rosolic Acid.*—The effect is doubtful. The color is probably deepened.

*Indol Production.*—Negative.

*Relation to Temperature.*—Grows at the temperature of the room and at 35° to 36° C.

1. Wright: Mem. Nat. Acad. Sc., 1894, vii, 422–474.
2. Chester: l. c., 311.

### PSEUDOMONAS CENTRIFUGANS (Wright) Chester

Reported by Wright[1] as *Bacillus centrifugans* variety a. Named by Chester.[2] Isolated from the water of the Schuylkill River. In the summer occurs abundantly both in the Schuylkill and Delaware water.

**Morphology.**—A medium-sized motile bacillus with rounded ends, sometimes in pairs and in long forms. It has a polar flagellum; no spore formation observed.

**Cultural Characters.**—*Colonies in Gelatin.*—After twenty-four to forty-eight hours the surface colonies are round, saucer-shaped depressions, containing faintly hazy, liquefied gelatin, several millimeters in diameter, which rapidly increase in size. About the center there may be a few grayish flocculi, while at the margin they are well defined by a grayish line. Under a low power the liquefied gelatin is granular in appearance, and a circulating motion may be seen in it, while scattered, dark, granular clumps, more closely gathered together about the center, may be sometimes observed. At the margin there is a fringe of short radiating fibrils. The deep colonies are rounded, dark, and granular, and may have a greenish shimmer. They soon become surrounded by a zone of liquefaction, and break through the gelatin to form colonies similar to those described above. When they are just beginning to liquefy they are subject to some variation in their appearance.

*Gelatin Stab.*—Liquefaction rapidly extends to the wall of the tube at the surface, and also all along the inoculation line, in a form varying from that of a funnel to that of a champagne glass. The liquefied gelatin is clouded, and when the liquefaction is well advanced along the line of inoculation a thick, whitish, flocculent mass is seen at the bottom. On the surface there is a delicate pellicle, and in completely liquefied cultures beneath this a greenish tint may be observed. The reaction of the liquefied gelatin is alkaline.

*Acid Gelatin.*—Grows less vigorously.

*Agar Slant.*—Semitranslucent, shining, grayish, rather thin stripe, with scalloped margins. In older culture becomes a dirty-brown color, while the agar acquires a marked brownish-green tint.

*Bouillon.*—Clouded. A broken, imperfect pellicle forms on the surface and the bouillon acquires a brownish-green tint.

*Potato.*—Variable, thick, widely spreading, dense growth, somewhat rough or granular on the surface; the color varies from cream gray to pinkish.

*Litmus Milk.*—Coagulated and decolorized; cloudy serum, containing flocculi; reaction is neutral or slightly acid.

*Sugar Gelatin in Deep Stab.*—Fair growth with some gas production.

*Rosolic Acid.*—Color deepened.

*Indol Production.*—Marked reaction; does not produce nitrites.

*Relation to Temperature.*—Grows at the temperature of the room and at 35° to 36° C.

1. Wright: l. c., 422–474.
2. Chester: p. 312.

### PSEUDOMONAS PROTEA Frost

Isolated by Frost from filtered Potomoc River water at Washington by the use of lactose bile and Endo's medium. Agglutinated by the serum of patients with typhoid fever and by the serum of animals immunized with *Bacillus typhosus.*

**Morphological and Cultural Characteristics.**—Straight rods, single or in short chains, varying from 1.2 to 2.5 $\mu$ in length; average length about 1.7 $\mu$; average thickness about 0.6 $\mu$; flagella always polar, usually a single flagellum from only one pole; forms with one flagellum at each pole, or with two flagella at one pole, are relatively rare, and probably represent two or more bacteria in close approximation.

*Motility.*—Young agar or bouillon cultures are actively motile; motility, however, is sometimes lost in old cultures, or in bouillon cultures frequently transplanted and kept at 37° C.

*Spores.*—Not observed.

*Staining.*—Stained readily and uniformly with watery anilin stains; decolorized by Gram's method.

*Agar Slant.*—Growth fairly abundant in twenty-four hours at 37° C.; slightly brownish-white, smooth, moist, soft, edges uniform, not spreading; growth becomes brownish after several days.

*Plate Colonies Agar.*—Not distinctive; small, round, white at end of twenty-four hours.

*Hesse's Agar.*—Colonies white, wide-spreading, edges becoming radiate.

*Lactose Litmus Agar.*—Clear, colorless, transparent, generally slightly larger and less clear than colonies of *Bacillus typhosus.*

*Endo's Medium.*—Clear, transparent, colorless, or slightly pinkish; generally larger and less clear than colonies of *Bacillus typhosus.*

*Gelatin* (12.5 per cent.). In twenty-four hours, very small, white; in forty-eight hours, 2 to 5 mm. in diameter, liquefying, round, cup-shaped, grayish with white center; older cultures often present a varying proportion of very slowly liquefying colonies.

On none of the above media are the colonies at all distinctive. On agar, lactose-litmus agar, and Endo's medium they may easily be mistaken for typhoid colonies.

*Glycerin Potato.*—Growth in twenty-four hours at 37° C., visible but scant; white, moist; becomes brownish after several days, but remains scant.

*Gelatin Stab.*—At 37° C., liquefied in twenty-four hours; at 20° C., liquefaction always takes place, forming a deep funnel-shaped depression; the rapidity of the liquefaction, however, varies greatly in different strains, and in the same strain at different times.

*Blood-serum.*—Liquefied with varying rapidity; usually noticeable in one to four days at 37° C.

*Bouillon.*—Uniform turbidity in twenty-four hours; reaction alkaline after a few days.

*Litmus Milk.*—Slight acidity in twenty-four hours, with formation of a semisolid clot; solution of the casein, complete or partial, at a very inconstant rate; terminal reaction (ten to twenty days) usually acid, sometimes alkaline. The initial acidity and coagulation of litmus milk are constant characteristics. Solution of the casein is a very inconstant characteristic. All strains when freshly isolated peptonized milk rapidly; after several months on artificial culture media this property is diminished in all strains, and varies greatly with different lots of litmus milk. The production of alkali is also inconstant. As nearly as can be ascertained, the production of a high degree of primary acidity in litmus milk retards or prevents peptonization; and subsequent alkali formation, if taking place at all in such cases, is insufficient to neutralize the acid already formed.

*Fermentation Tube.*—Dextrose bouillon, fermented with production of acid and 10 to 30 per cent. gas in twenty-four hours; saccharose bouillon, neither acid nor gas; lactose bouillon, neither acid nor gas; growth in closed arm in all the above media.

*Peptone Solution* (1 per cent.).—Indol reaction very marked in four days.

*Nitrate Bouillon.*—Nitrites and ammonia formed.

*Odor.*—Disagreeable, putrefactive.

*Optimum Temperature.*—Growth most rapid at 37° C. Grows readily but less rapidly at 20° C.

*Pathogenic Action.*—Virulent to guinea-pigs, ½ c.c. twenty-four-hour broth cultures injected intraperitoneally producing acute fibrinous peritonitis fatal in thirty hours, the organism being recovered from the peritoneal exudate and heart's blood. The sterile filtrates of twenty-four- and forty-eight-hour broth cultures are also toxic and occasionally fatal to guinea-pigs of 300-gram weight in doses of 2 c.c. amounts, where injected intraperitoneally. The lesions shown at autopsy consist of intense enteritis and peritonitis.

Frost: Hyg. Lab. Bull., Pub. Health and Mar. Hosp. Serv., 1910, No. 66, 29.

### PSEUDOMONAS SINUOSA (Wright)

Described by Wright as *Bacillus sinuosus.* Isolated from the water of the Schuylkill River. At one time not uncommon.

**Morphology.**—A medium-sized, short, motile bacillus, with rounded ends, occurring often in pairs and sometimes in longer forms. It has a polar flagellum; some individuals probably have 2 to 4 flagella; no spore-formation observed.

**Cultural Characters.**—*Colonies in Gelatin.*—On the second or third day the surface colonies are very thin, delicate, grayish, translucent and opalescent expansions, with very irregular, sinuous outlines, and of a diameter of several millimeters, if the colonies are not too numerous. Under a low power they are thin, translucent, very irregular and sinuous in outline, and show various waving and irregular lines, especially near the margins, which suggest the appearance of wood graining. Sometimes they are brownish toward their centers. If the colonies are widely separated they may attain a diameter of 6 mm. or more, and in such a large colony delicate radiating foldings have been observed running from a faint whitish nucleus toward the periphery. The outline in the large colonies is extremely irregular and sinuous, and they remain thin and translucent, while under the low power they are seen to have lost the "wood graining" appearance, and are finely granular. The deep colonies under a low power are finely granular and brownish, with rounded or slightly irregular, sharply defined outlines.

*Gelatin Slant.*—Grayish-white, smooth, shining, semitranslucent stripe, with sharply defined, irregular margins; does not spread widely.

*Acid Gelatin.*—Grows well.

*Agar Slant.*—Narrow, grayish-white, thin, shining, semitranslucent stripe, with slightly scalloped margins.

*Bouillon.*—Clouded and has a whitish, stringy sediment; no pellicle on surface.

*Potato.*—Dirty brown-gray, moist, not thick, rather rough appearing growth, which spreads rather widely.

*Litmus Milk.*—Pink, white at bottom; no coagulation after six weeks at the temperature of the room; reaction is acid.

*Sugar Gelatin in Deep Stab.*—A thick cord of confluent whitish colonies forms along the line of inoculation, and there is an abundant production of gas.

*Rosolic Acid.*—Color is deepened.

*Indol Production.*—A faint reaction; the addition of nitrite solution is necessary.

*Relation to Temperature.*—Grows well at the temperature of the room and at 35° to 36° C.

Wright: l. c., 422.

## FAMILY III. SPIRILLACEÆ Migula

Cells elongate, more or less spirally curved. Cell division always transverse, never longitudinal. Cells non-flexuous. Usually without endospores. As a rule motile by means of polar flagella, sometimes non-motile. Typically water forms, though some species are intestinal parasites.

### GENUS 1. VIBRIO Mueller

Cells short bent rods, rigid, single or united into spirals. Motile by means of a single (rarely two or three) polar flagellum, which is usually relatively short. Many species liquefy gelatin and are active ammonifiers. Aërobic and anaërobic. No endospores. Usually Gram-negative. Water forms, a few parasites.

*Vibrios found in man*

Liquefying

Type species *Vibrio comma* (Koch) Schröter.
" *Massauah* Pfeiffer.
" *Gindha* Pfeiffer.
" *proteus* (Finkler-Prior) Buchner.
" *septicus* Kolle.
" *sputigenus* (Migula).
" *Milleri* (Migula).
" *lingualis* (Migula).

Non-liquefying

" *Wolfii* (Migula).
" *nasalis* (Migula).
" *tonsillaris* Stephens and Smith.
" *surati* (Laub and Paton).

*Vibrios found in pigeons*

*Vibrio Metchnikovi* Gamaléia.

*Vibrios found in swine or swine dung*

*Vibrio Smithii* (Migula).
" *subtillissimus* (Migula).
" *suis.*
" *hyos.*
" *intermedius* (Migula).
" *grossus* (Migula).

*Vibrios found in cattle*

*Vibrio fetus* Smith and Taylor.

*Vibrios found in mussels*

*Vibrio cardii* Klein.

*Vibrios from fishes*

Bergman's vibrio from keratomalacia.
*Vibrio anguillarum* Bergman.

*Vibrios from cheese*

*Vibrio tyrogenus* (Deneke).

*Vibrio found in water*

Liquefying

*Vibrio aquatilis* Günther.
" *marinus* (Russell).
" *Berolinensis* Neisser.
" *Danubicus* Heider.
" *Schuylkilliensis* Abbott.

Non-liquefying

" *Bonhoffi* (Migula).
" *portuensis* (Migula).

*Vibrios from hay infusion*

*Vibrio percolans* Mudd and Warren.

*Pigmented vibrios*

Yellow

*Vibrio aureus* Weibel.
" *flavus* Weibel.

Red

*Vibrio saprophiles* (Weibel).

Anaërobes

Mühlens anaërobic vibrio.
*Vibrio tenuis* Veillon and Repaci.
Soper's vibrio.
Tunnicliff's vibrio.

**VIBRIO COMMA (Koch) Schröter**

Discovered in the dejecta of patients with Asiatic cholera by Koch[1] in 1883 and established as the cause of the disease. Known also as *Spirillum choleræ asiaticæ*, *Vibrio choleræ asiaticæ*, the comma bacillus, etc.

**Morphology.**—Short, slightly bent organisms looking like the German mark of punctuation known as the comma. On agar cultures twenty-four hours old they usually measure 0.3 to 0.4 by 2 to 3 $\mu$. They may also be longer and thicker, measuring 0.5 by 3 to 4 $\mu$. They are disposed as single individuals, half-circles, S-shaped and long screws or spirals measuring 0.35 by 5 to 8 $\mu$. In old cultures these long screws or spirals are more common. The turns of the screw may be almost lost so that the organisms look like straight threads. In old cultures also distorted forms of various shapes are common, the so-called involution forms. The individual cells are thicker, almost straight, swollen, spherical, often showing light areas[1] in the interior. Spindle-shaped organisms and bladder-like forms also occur. These distorted structures were called arthrospores by Hueppe.[2] They are not true spores, but probably degeneration products. Often the material in the culture consists almost entirely of granules. On dextrose agar in twenty-four hours involution forms are numerous. They are long and swollen, measuring 0.7 by 3 to 4 $\mu$ or 0.5 by 5 to 8 $\mu$. Some forms appear as swollen spirals, measuring 1 to 1.5 by 15 to 17 $\mu$, while others are irregular and much distorted.

In strains kept a long time on artificial media the typical morphology of the cholera vibrio is lost and the organisms look like straight rods. The morphology may be restored by animal passage.

In the dejecta of cholera patients the organisms appear in enormous numbers, often in almost pure cultures. In this material long spirals are more frequent than in cultures.

In the tissues the characteristic form is not seen, the organisms being nearly straight like the glanders bacillus.

**Staining Reactions.**—The cholera vibrios stain by the ordinary basic anilin dyes, best by carbolfuchsin diluted 1 to 10. Gram-negative. By staining with methylene-blue, decolorizing with acid alcohol (HCl), and counterstaining with weak pyronin dark granules appear in a bluish-red body (Schauffler). Involution forms stain irregularly, the swollen bladder forms very faintly. In the tissues the best results are obtained with methylene-blue and differentiation in dilute acetic acid alcohol.

**Motility** is extremely active, the organisms shooting violently from one place to another. When a mass of organisms is examined it seems to be in violent agitation, compared by Koch to the swarming of gnats. Motility due to a single terminal flagellum demonstrated by Loeffler's or van Ermengem's stain. Kolle and Schürmann[3] state that the multiple terminal flagella reported by some authors were found on cholera-like vibrios, not on the true cholera vibrio.

**Cultivation.**—The cholera vibrio can be cultivated on a great variety of media, growing best on those with an alkaline reaction like neutral agar or gelatin, to which 3 c.c. of 10 per cent. sodium hydrate are added to each 100 c.c. It does not grow on slightly acid gelatin, 0.07 per cent. HCl in the media preventing development (Kitasato). It is an obligate aërobe, not growing at all in the complete absence of oxygen (Hesse). Growth most abundant at 30° to 40° C. Very slow development at 8° to 16° C. Excellent growth at 22° to 25° C.

Fig. 27.—*Vibrio comma* from plain agar, twenty-four hours old, stained with gentian-violet.

**Cultural Reactions.**—*Plain Agar Plates.*—Surface colonies appear in twenty-four hours, measuring 0.5 to 1 mm. in size. In forty-eight hours they are larger, measuring 1 to 1.5 mm., round, regular, and uniform. Under low power they are round and regular with dense centers and thin margins with entire edges. Others may be deep colonies which have broken through the surface and spread somewhat. Some colonies are distinctly moruloid with daughter colonies about them. Deep colonies punctiform, under low power round, regular, and corrugated, or biconvex disks. The superficial colonies grow rapidly, become thick, heaped-up in the center, measuring 2 to 4 mm. in five days and 0.5 to 0.75 cm. in ten to twelve days. The deep colonies increase in size somewhat more slowly and usually appear as distinct biconvex disks to the naked eye.

*Plain Agar Slant.*—Thick, moist, abundant growth in twenty-four hours. It increases rapidly, becomes thicker and more abundant, and in three to four days is distinctly yellowish in color. In eight to ten days the growth is quite thick and heavy, often brownish.

*Plain Agar Stab.*—Abundant line growth, some surface growth in twenty-four hours. Both line and surface growth increase rapidly and in four to five days the surface growth is abundant and yellowish. Later, in ten to twelve days, this extends to the wall of the tube and is distinctly brown in color.

*Dextrose Litmus Agar.*—Surface colonies appear small in twenty-four to forty-eight hours, round, regular, opaque, and somewhat raised. They measure 1 to 2 mm. in diameter

and frequently show reddish areas in the center from the accumulation of acid. Under low power the colonies are round and regular with dark central areas and thin margins with entire edges. Deep colonies almost pin-point, under low power round and regular or oval and biconvex disks. When the plates are closely seeded the agar is quite acid in reaction. Where the colonies are isolated they may appear red from acid, while the surrounding agar remains neutral or alkaline. Older surface colonies become much larger, 0.5 to 0.75 cm. in diameter, with a much corrugated or folded surface. In old plates the acidity may be overcome and the agar become neutral or alkaline.

*Dextrose Litmus Agar Slant.*—Growth sparse at first, appearing only in two to three days. The agar has a definite acid reaction. The growth becomes somewhat heavier in eight to ten days, but remains moderate. The acidity persists in the agar for some time and is only overcome in very old cultures, fifteen to twenty days.

*Gelatin Plates.*—Colonies grow very slowly, appearing only at the end of two to three days, very small and fine, measuring 0.25 mm., and lying in small areas of liquid gelatin. Under low power the colonies are round, regular, and uniform, with a zone of liquid gelatin about them. When the plates are crowded with colonies liquefaction is very rapid, the gelatin

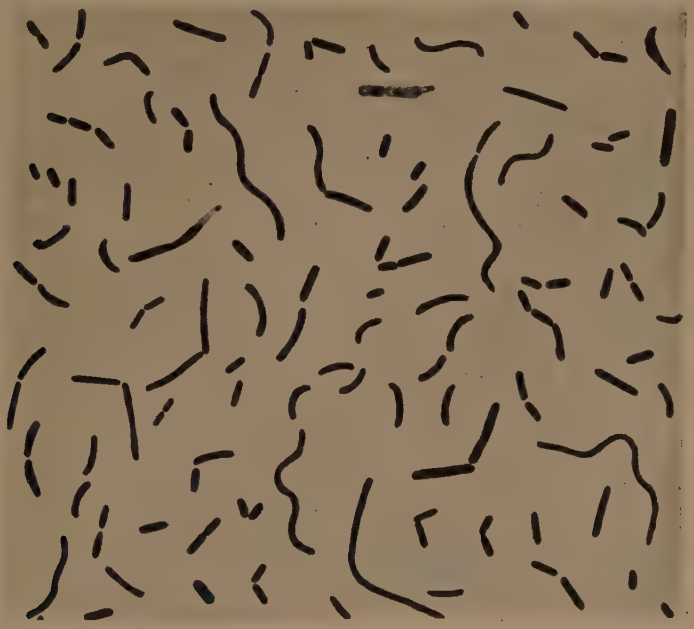


Fig. 28.—*Vibrio comma* from dextrose agar, four days old, stained with gentian-violet.

being quite fluid in four to five days. Where the colonies are isolated they become larger, in five to six days measuring 1 to 2 mm., lying in zones of liquid gelatin 0.25 cm. in diameter. Under low power the colonies are woolly with feathery edges. Later the original colonies may be surrounded by zones of daughter colonies which lie in the liquid gelatin surrounding the original growth. Liquefaction is nearly always complete by the end of ten days.

*Gelatin Stab.*—Growth begins at the surface, a slight cupping appearing in two to three days containing a little liquid and turbid gelatin. There is little or no growth along the line of inoculation. In four to five days the liquefaction is more marked and in eight to nine days a well-developed cone-shaped liquefaction appears. The liquid gelatin is turbid and has a whitish granular sediment at the bottom. Liquefaction is usually complete in twelve to fifteen days.

*Blood-serum.*—Fairly abundant thin yellow growth in twenty-four to forty-eight hours. The growth increases and slowly sinks into the serum. In eight to ten days the growth is quite abundant, sunken along the entire line of inoculation, the serum being softened somewhat in the upper portion. In old cultures there is considerable softening and occasionally there is a very definite liquefaction of the serum.

*Potato.*—Growth usually thin and scanty, whitish.

*Broth.*—Rapid development, dense turbidity, faint sediment, and heavy scum in twenty-four hours. The turbidity and sediment increase and in three to four days they are very

marked, while a very thick heavy scum develops on the surface. In older cultures the scum may precipitate and form part of the heavy sediment.

*Peptone.*—Growth as rapid, or even more rapid, than in broth, turbidity, scum, and sediment appearing in twenty-four hours and increasing rapidly. In cultures eight to ten days old the turbidity is dense, the sediment abundant, and a very heavy scum develops on the surface. In old cultures this scum may be precipitated.

*Milk.*—Growth rapid, with slow development of an acid reaction. The acidity is marked in four to five days, remains high for a long period, but is not usually sufficient to coagulate the milk even on heating.

*Dextrose Fermentation Tube.*—Turbidity in both arm and bowl with some sediment in twenty-four hours. The turbidity and sediment increase. Reaction highly acid in three days. In *saccharose* the growth characters are the same and the reaction acid. In *lactose* a heavy scum develops on the surface as well, with an acid reaction.

Other media for the cholera vibrio are those of Dieudonné,[4] Deeleman,[5] and Teague. Some of these are elective for this organism.

**The Cholera Red Reaction.**—On the addition of C. P. $H_2SO_4$ or HCl to broth cultures a red color develops as first shown by Poehl[6] and by Dunham.[7] The substance giving the red color is nitroso-indol. True cholera vibrios form indol and reduce nitrates to nitrites. The reaction is not absolutely diagnostic, however, since closely related vibrios also give the reaction. Nitroso-indol is best demonstrated in 1 per cent. peptone solution containing 0.5 per cent. NaCl.

**Resistance.**—The cholera vibrio is very sensitive to drying, high temperatures, and to the growth of other organisms. Broth cultures dried on coverslips are dead in two hours (Koch). Destroyed rapidly by sunlight. Destroyed by boiling almost instantly, at 80° C. in five minutes, at 56° C. in half an hour (Kolle and Schürmann). When dried on silk threads in a desiccator killed in two to four days (Kitasato). Killed by Roentgen rays in twenty to thirty minutes (Rieder). Destroyed easily by disinfectants (Koch and Gaffky[8]), by 1 per cent. phenol in five minutes, by 0.5 per cent. in ten minutes. Killed by sublimate solution containing traces of mercuric chlorid in strength of 1 to 2 to 3,000,000 in five to ten minutes (Forster). Killed in a few seconds by HCl or $H_2SO_4$ in a strength of 1 to 10,000. Very sensitive to chlorin, 1 part to 1,000,000 parts of water destroying them in fifteen minutes (Harding). Destroyed in the dejecta in an hour by the addition of milk of lime till an alkaline reaction appears (Pfuhl).

Dies out rapidly in distilled water, but may survive several weeks in the water of streams and pools. Cultivated by Wernicke[9] from the mud of an aquarium after months. Fails to survive in contact with putrefactive bacteria. Not obtained from sewage by Koch after twenty-four to thirty hours. Not found usually in foul dejecta after one to three days (Dunbar). Rarely found, however, up to thirty days. No survival on clothing except for a short time. Survive in sterile milk ten days or more, but only one to two days in unsterilized milk, being destroyed apparently by the acids developing. Survive only a short time on food. In weak tea, 4 parts to 100 parts water, survive eight days, in strong tea, 4 parts to 100, die out in an hour (Friedrich). Die out in wine in half an hour, in beer in three hours.

**Pathogenic Action.**—Animals are not infected by the cholera vibrio under natural conditions (Gaffky). Freshly isolated cultures are virulent to small animals like guinea-pigs and rabbits, but there is great variation in the virulence of different strains and the type of infection depends largely upon the mode of administration. Koch produced fatal infection in guinea-pigs by making the gastric juice alkaline by the administration of 5 c.c. soda solution (5 per cent.), injecting tincture of opium to control the movements of the intestine, and introducing the vibrios directly into the stomach. The animals died in twenty-four to thirty-six hours. Other vibrios, like those of Finkler-Prior and Deneke, and *Vibrio septicus* of Kolle, may produce the same effect when introduced in this way.

Intraperitoneal injection produces infection in guinea-pigs with regularity (Koch) and this method suffices to estimate the virulence of cultures with great exactness (Pfeiffer[10]). At autopsy a peritonitis is found with the organisms present in large numbers. The usual dose for freshly isolated cultures is $\frac{1}{10}$ to $\frac{1}{20}$ of an oesa for animals of 200-gram weight. With large doses the organisms may appear in the blood (Sobernheim[11]). With doses just large enough to produce the death of the animal the organisms may be absent from the peritoneum. The internal organs show no changes and only rarely are organisms present in the intestines.

In rabbits, especially in young animals, direct injection into the ear vein produces a fatal infection. Thomas[12] says that the intestinal wall shows lesions like those in cholera. A few drops only of a 1 : 1,000,000 dilution of a pure culture suffice to produce a fatal infection and the animals may show a characteristic diarrhea during life (Issaeff and Kolle[13]). Similar effects have been produced in young rabbits without the diversified intestinal flora of the adult by Metchnikoff,[14] and in the Zieselmouse, *Spermophilus guttatus*, by Sabolotny.[15] Fatal infection was also produced in rabbits by Nicati and Rietsch,[16] who opened the abdominal cavity, tied the common bile-duct, and introduced pure cultures directly into the duodenum. The organisms multiplied in the small intestine and in the epithelial cells.

Pigeons are almost entirely immune to the cholera vibrio, enormous numbers being re-

quired to produce infection when injected directly into the breast muscles. This is a differential criterion of great value in separating the cholera vibrio from *Vibrio Metchnikovi*.

In large animals artificial infection is much more difficult. According to Richards swine fed on cholera dejecta die in fifteen minutes to two and a half hours, apparently from toxemia.

The virulence of cultures varies greatly, ranging from $\frac{1}{2}$ to $\frac{1}{20}$ of an oesa as a fatal dose. Weakly virulent cultures become highly virulent on animal passage, and highly virulent cultures retain their virulence by the same procedure. According to Paltauf the virulence may be increased by cultivation in cholera immune serum.

While Asiatic cholera has never been produced purposely in man, a number of laboratory workers have become infected while working with the organism, and in one instance at least the infection resulted fatally (Dr. Oergel in Hamburg).

**Toxins.**—The symptoms of Asiatic cholera, especially in the algid stage, point to a severe intoxication with poisons from the organism and many attempts have been made to obtain these poisons. It has been shown by Pfeiffer that filtrates of young cultures were little or non-poisonous, while filtrates of cultures several months old were intensely poisonous, not producing the symptoms and lesions found in cholera however. It is now usually believed that the toxicity of such old cultures is to be attributed to some kind of putrefactive product. Fresh agar cultures killed by heat (56° C., one hour) are intensely poisonous, especially to guinea-pigs. These primary poisons are labile and according to Pfeiffer and Wassermann[17] can be converted into secondary poisons, less active, by chemicals and by heating to 80° to 90° C. Such poisons are properly considered endotoxins, closely tied to the plasma of the cells and liberated by their distintegration. Similar endotoxins, poisonous to rabbits, have been demonstrated by Gamaleía.[18] The primary poison is regarded by Gamaleía and by Pfeiffer as a nucleo-albumin, the secondary as a nuclein. Finally Kraus[19] has found soluble poisons acting upon hens, pigeons, guinea-pigs, and mice in a few true cholera vibrios (El Tor strains) which also produce hemolysins. No antitoxin to these soluble poisons has been produced.

**Hemotoxins.**—The capacity of the cholera vibrio to dissolve blood-corpuscles was first observed by Koch in gelatin containing blood and by Eijkman[20] in agar media containing rabbit's blood. It has now been shown that this capacity varies greatly and that some strains, known as Nasik, El Tor, etc., produce hemotoxins in great abundance. According to Kraus an alkaline medium is necessary for their production, best obtained by adding 2 to 4 c.c. of a 5 per cent. sodium hydrate solution to 1000 c.c. ordinary broth. The hemolysins are demonstrable on the second to the fifth days and have a maximum strength of about 0.1 c.c. in unfiltered cultures and about 0.5 c.c. in filtered material. Hemotoxins for several different kinds of corpuscles, rabbit, ox, goat, and wethers, may be present in the same filtrate. The hemotoxins may also be demonstrated on blood agar plates. Many true cholera vibrios fail to produce hemotoxins. Kolle and Schürmann regard the capacity to produce hemotoxins as normal to the cholera vibrio and to certain other vibrios, those strains which fail to produce them being possibly mutants.

**Rapid Cholera Diagnosis.**—For practical reasons of quarantine various methods of obtaining a rapid diagnosis in suspected cases of cholera have been devised. The careful examination of material from stools, especially the flecks of mucus, often reveal characteristic comma bacilli. The material may be cultivated in Dunham's peptone solution, in which they grow rapidly at the surface, in gelatin in which characteristic colonies appear, or on Dieudonnè's elective medium. Pure cultures should be identified culturally and by serological reactions, agglutination, bacteriolysis (Pfeiffer's phenomenon), complement fixation, etc.

**Vaccination.**—Active immunity against the cholera vibrio can be produced by a variety of methods, living virulent cultures, living non-virulent cultures, dead cultures, filtrates, and extracts of the organisms. The sera of vaccinated animals contains agglutinins, bacteriolysins, precipitins, complement-fixing substances, opsonins, etc. Both vaccines and sera have been employed on a large scale in prophylaxis, the vaccines with a considerable degree of success.

1. Koch: Deutsche med. Wchnschr., 1883, ix, 615. *Ibid*, x, 725.
2. Hueppe: Fortschr. d Med., 1885, iii, 619.
3. Kolle and Schürmann: *In*: Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1912, iv, 1.
4. Dieudonné: Centralbl. f. Bakteriol. [etc.], 1909, l, 107.
5. Deeleman: Arb. a. d. K. Gsndhtsamte, 1897, xiii, 374.
6. Poehl: Ber. der Deutsch. chem. Gesellsch., 1886.
7. Dunham: Ztschr. f. Hyg., 1887, ii, 337.
8. Koch and Gaffky: Arb. a. d. K. Gsndhtsamte, 1887, iii, 1.
9. Wernicke: Hyg. Rundschau, 1895, v, 736.
10. Pfeiffer: Ztschr. f. Hyg., 1893, xiv, 485. *Ibid*, 1894, xvi, 268.
11. Sobernheim: Hyg. Rundschau, 1893, iii, 997.
12. Thomas: Arch. f. exper. Path. u. Pharm., 1893, xxxii, 38.
13. Issaeff and Kolle: Ztschr. f. Hyg., 1894, xviii, 17.

14. Metchnikow: Ann. de l'Inst. Pasteur, 1894, viii, 529.
15. Sabolotny: Centralbl. f. Bakteriol [etc.], 1894, xv, 150.
16. Nicati and Rietsch: Deutsche med. Wchnschr., 1884, x, 634. *Also*: Transl. Arch. d. physiol. norm. et path., 1885, 3. s., vi, 72.
17. Pfeiffer and Wassermann: Ztschr. f. Hyg., 1893, xiv, 46.
18. Gamaleía: Arch. de méd. expér. et d'anat. path., 1892, iv, 173.
19. Kraus: Wien. klin. Wchnschr., 1902, xv, 120.
20. Eijkman: Centralbl. f. Bakteriol. [etc.], 1901, xxix, 841.

### Vibrio Massauah Pfeiffer

Found by Pasquale[1] in Massauah and named by Pfeiffer.[2] Isolated by Pasquale from the dejecta of a patient with cholera-like symptoms. Not definitely associated with a small epidemic of cholera prevailing at the time.

Differentiated from the cholera vibrio by the presence of several up to 4 flagella and not the single flagellum of Koch's organism. Gelatin colonies also are different. They are quite round, sharply outlined, yellowish disks. Liquefaction is slow and incomplete, nitroso-indol reaction in peptone solution positive. More virulent than the cholera vibrio and like *Vibrio Metchnikovi*. Pigeons and guinea-pigs are killed by subcutaneous and intramuscular injections. Young rabbits develop cholera-like symptoms from feeding. Probably pathogenic for man. Differentiated also from the cholera vibrio by immunity reactions.

1. Pasquale: Gior. med. d. r. esercito, 1891.
2. Flügge: Die Mikroorganismen. 1896, ii, 589.

### Vibrio Gindha Pfeiffer

Isolated by Pasquale[1] from the water of a spring at Gindha, near Massauah. Named by Pfeiffer.[2]

Fairly long, definitely curved rods, smaller than the cholera vibrio. They have single terminal flagella. Cultural characters like those of the cholera vibrio. Nitroso-indol reaction in peptone solution, twenty-four hours at 37° C., either very weak or not present. Not infectious for pigeons or guinea-pigs. Small doses of living or killed cultures kill guinea-pigs by intraperitoneal inoculation by intoxication. Separated from the cholera vibrio by immunity reactions.

1. Pasquale: Gior. med. d. r. esercito, 1891.
2. Pfeiffer: *In:* Die Mikroorganismen (Flügge). 1896, ii, 590.

### Vibrio proteus (Finkler-Prior) Buchner

Isolated as the *Kommabacillus der Cholera nostras* by Finkler and Prior.[1] Named by Buchner.[2] Present description from a culture from the American Museum.

**Morphology.**—On plain agar twenty-four hours old the organisms appear as short curved vibrios about 0.4 $\mu$ in width and 1.5 to 3 $\mu$ in width and 1.5 to 3 $\mu$ in length. Occasionally distinct spirals are formed by the juxtaposition of several individuals. Long forms also occur, measuring 7 to 10 $\mu$ in length and short forms measuring only about 1 $\mu$ in length. On dextrose agar all sorts of involution forms develop in twenty-four to forty-eight hours. Swollen organisms measuring 2 $\mu$ in width and 3.5 to 4 $\mu$ in length are common, either pointed or pear-shaped. Spirals 7 to 8 $\mu$ in length are also occasionally seen, together with plump rods about 2 $\mu$ in width and 3.5 to 4 $\mu$ in length, small oval bodies measuring 1.8 to 2.5 $\mu$ and large ovoid bodies measuring 3 by 3.5 $\mu$.

*Motility* very active, due to polar flagella.

*Stained* by the usual anilin dyes, best probably with dilute carbolfuchsin. Gram-negative.

**Cultural Reactions.**—*Plain Agar Plates.*—Growth is slow, colonies appearing in forty-eight hours. The superficial colonies measure 2 to 4 mm. in size. They are round, regular, with dense centers and thin margins. Under low power they show central nuclei which may

Fig. 29.—*Vibrio proteus* from plain agar twenty-four hours old, stained with gentian-violet.

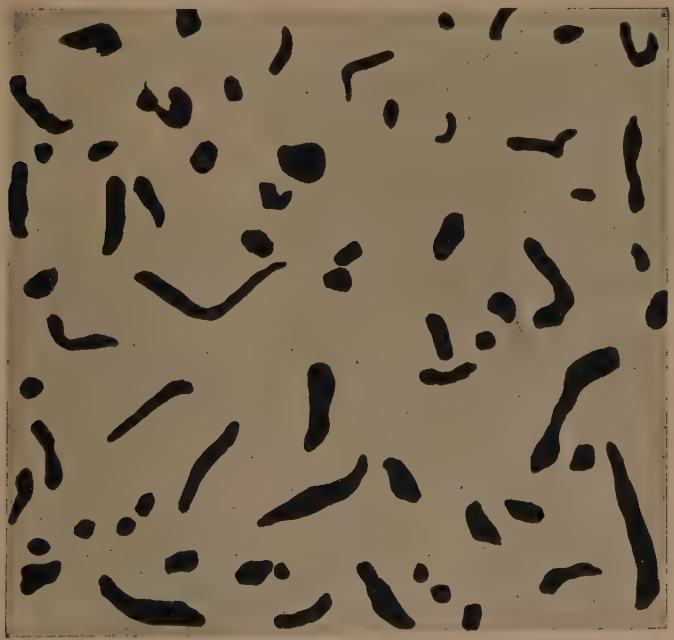


Fig. 30.—*Vibrio proteus* from dextrose agar twenty-four hours old, stained with gentian-violet.

be the original deep colonies or the centers may be merely thicker. The deep colonies are punctiform. Under low power they are round or oval, granular. In three to four days the superficial colonies are about $\frac{1}{2}$ mm. in size. The deep colonies are mostly punctiform, but may be rarely biconvex. In five to six days the superficial colonies are large and thick, $\frac{1}{2}$ to

¾ cm. in size, somewhat brown; they have dark centers and thin edges. The deep colonies are round or oval. In nine to ten days the colonies are large and spreading with thin, but entire margins, ¾ to 1 cm. The deep colonies are now definite biconvex disks. In old plates the colonies tend to become dark brown in color.

*Plain Agar Slant.*—In twenty-four hours a fairly heavy, non-spreading growth develops. In forty-eight hours this is thicker and more abundant, non-spreading. In three to four days the growth is somewhat brownish. In eight to ten days the growth is much thicker and more abundant, distinctly brownish in color.

*Plain Agar Stab.*—In twenty-four hours there is a faint growth along the line of inoculation, and some surface growth. In forty-eight hours the growth is abundant along the line and still slight on the surface. In three days there is considerable line and surface growth. In eight to ten days the line and surface growth are abundant. In old cultures the surface growth extends to the edge of the tube.

*Dextrose Agar Plates.*—In twenty-four hours the superficial colonies measure ½ to 1 mm. and are round, regular, rather thin. The deep colonies are pin-point, the bottom colonies translucent, and thin. Under low power the superficial colonies have a peculiar folded granular appearance, margins irregular, not spreading, somewhat translucent. The centers are folded into ridges, the peripheries granular. Other colonies are round, regular, and granular. Under low power the deep colonies are round, oval, or biconvex disks. The bottom colonies are thin, spreading, but the margins are entire. Reaction of the plates acid. In forty-eight hours the colonies are 2 mm. in diameter, show dark centers and peculiar jagged outlines. In three days the colonies are larger, ½ cm. in size, showing irregular edges and dark centers. The acidity is less marked. In ten to twelve days the colonies are very characteristic. They vary from 1 mm. to ½ cm. in size, have heaped-up dark greenish centers and thin margins. Under low power they are quite opaque with entire edges. Other colonies are 1 to 1½ cm. in size, the surface is heaped-up and corrugated like "mesentericus" cultures. The plates are all alkaline in ten to twelve days.

*Dextrose Agar Slant.*—In twenty-four hours a faint growth occurs with a slight acidity. In forty-eight hours the growth is increased and the reaction more marked. In three to five days there is a fairly abundant growth with considerable acidity. The acidity may remain permanently or slowly give way to an alkaline reaction.

*Broth.*—Turbidity, some sediment, no scum in twenty-four hours. In forty-eight hours the turbidity and sediment are increased, but no scum develops. In older cultures the turbidity and sediment are marked without scum formation.

*Peptone.*—Turbidity and sediment like that in broth. No scum.

*Litmus Milk.*—Slight acidity, developing in eight to ten days. Some coagulation on heating.

*Potato.*—No growth.

*Blood-serum.*—Considerable growth in twenty-four hours and marked sinking at the line of surface inoculation. Gelatin brownish, water of condensation turbid. In forty-eight hours the liquefaction is more marked with the water of condensation turbid. In four to five days the serum is half liquefied. In ten days the serum is almost completely liquefied and in old cultures it collects as a semifluid mass.

*Gelatin Stab.*—In twenty-four hours growth along line of inoculation with a beginning liquefaction. In forty-eight hours the line growth is increased. There is a distinct cupping of the surface. In three days the growth is marked along the line of inoculation and at bottom of the tube and there is considerable surface cupping. In five days the cupping is increased. In ten days half of the gelatin is liquid and in fifteen days it is almost entirely liquefied.

*Gelatin Plates.*—Slow growth, colonies appearing in forty-eight hours as small white dots, ½ mm. in diameter, lying in areas of liquid gelatin. These colonies are distinctly hazy. Under low power the colonies are woolly with feathery edges; each colony sits in a mass of liquid gelatin. In three days the colonies are larger, ½ to 1 mm. in size, feathery, the separate colonies lying in zones of liquid gelatin. In four days these zones are increased in size and the colonies have very hazy, fuzzy outlines. In eight days the colonies are granular with many marginal daughter colonies. The zones of liquid gelatin are now 2 cm. wide. By ten days all the plates are liquefied.

**Fermentation Tubes.**—*Dextrose.*—In twenty-four hours the bulb is turbid, no scum, no sediment, arm clear. Granules appear along the side of the tube in the arm. In forty-eight hours a precipitate develops in the arm. In three days the bulb is fairly turbid, arm clear, with some precipitate, no scum. Acidity high.

*Saccharose.*—In twenty-four hours turbidity in bulb, no scum, no sediment. Granules along side of the arm, which remains clear. In forty-eight hours the turbidity is increased and the granules are precipitated along the side of the arm, which is otherwise clear. No scum. In three days increased turbidity, some sediment, arm clear, no scum. Moderate acidity.

*Lactose.*—In twenty-four hours turbidity in arm and bowl, no scum, some sediment. Granules along the side of the arm. In two to three days turbidity in arm and bowl increased, no scum, slight sediment. Moderate acidity.

Nitroso-indol reaction not given.
Pathogenicity for guinea-pigs less than that of cholera vibrio.

1. Finkler and Prior: Deutsche med. Wchnschr., 1884, x, 579. *Ibid*, 1884, x, 632.
2. Buchner: Sitzungsb. d. Gesellsch. f. Morphol. u. Physiol. in München. i, p. 21.

### VIBRIO SEPTICUS Kolle

Found by Kolle in cholera nostras.

**Morphology** and *cultural reactions* resemble those of the cholera vibrio, but the organism is not agglutinated by cholera serum, and does not give the Pfeiffer reaction with it. Not especially pathogenic for pigeons, but intensely virulent for guinea-pigs. According to Kolle this organism kills guinea-pigs more rapidly and with smaller doses than any other known pathogen. One drop of a greatly diluted culture when applied to a wound of the skin of a guinea-pig kills the animal of septicemia in one to four hours.

Kolle and Schürmann: *In:* Handb. d. path. Mikroorgan. (Kolle and Wassermann). 2. Aufl., 1912, iv, 1.

### VIBRIO SPUTIGENUS (Migula)

Isolated by Brix[1] from the sputum of a pneumonia patient and called by him "Vibrio from sputum." Named by Migula[2] *Microspira sputigena.*

**Morphology.**—Like the cholera vibrio in length and thickness. Frequently forms spirals, especially in broth, consisting of three to four organisms.

*Motility* active due to a single terminal flagellum.

*No spores.*

*Stains* by the usual basic anilin dyes, especially with carbolfuchsin. Decolorized by Gram's stain. Flagella stained by Loeffler's method.

**Cultural Characters.**—*Gelatin colonies* appear in twenty four hours at a temperature of 22° C. as fine points which give the plate an appearance as if it had been stuck with a needle. Under low power round finely granular yellowish disks. The colonies often show dark centers with concentric rings. In forty-eight hours the colonies reach the size of pin heads, the gelatin is fluid and shows in the middle a grayish-white thick bacterial mass surrounded by a turbid fluid. Under low power these colonies are brownish crumbled disks surrounded by a narrow lighter recticulated zone. In seventy-two hours the colonies attain the size of a hemp seed, in ninety-six hours the size of a lentil. By this time the plates are entirely fluidified. Deep colonies are originally round and sharply limited. Later they sink into the gelatin and break through to the surface. The development of the colonies is more rapid than the development of the cholera vibrio.

*Gelatin stabs* show depression the size of a lentil on the surface and a fine funnel-shaped growth along the line of the inoculation in twenty-four hours at 22° C. In seventy-two hours the superficial growth is the size of a pea. In eleven days the gelatin is fluidified across the top to a depth of $\frac{3}{4}$ to 1 cm. The fluid is clear and at the bottom has a deposit of crumbling yellowish-white masses of bacteria. A film develops on the surface. This vibrio liquefies the gelatin more rapidly than the cholera vibrio but more slowly than Finkler-Prior. The liquefied gelatin has a sweetish aromatic odor and an alkaline reaction.

*Agar Slant.*—Smooth, glistening, grayish-white moist layer, occasionally brownish. Surface development only in 1 per cent. peptone solution and in broth at 37° C., the fluid in the depths remaining clear. The surface scum is not so characteristic as that of the cholera vibrio.

*Potato.*—Slow growth at 22° C., more rapid at 37° C., producing a thin, soft, bright gray mass spreading laterally.

*Milk* acidified and clotted by the production of lactic acid.

Development only aërobic.

No nitroso-indol reaction.

Non-pathogenic.

1. Brix: Hyg. Rundschau, 1894, iv, 913.
2. Migula: System der Bakterien. 1900, ii, 981.

### VIBRIO MILLERI (Migula)

Originally described by Miller[1] from carious teeth. Named *Microspira Milleri* by Migula.[2] Not the organism usually found in the normal mouth. Present description from a culture from the American Museum.

**Morphology.**—On plain agar in twenty-four hours the organisms are plump, slightly curved and measure about 0.4 $\mu$ in diameter and 1.5 to 2.5 $\mu$ in length. Straight forms also occur, measuring about 0.4 $\mu$ in width and 1 to 1.5 $\mu$ in length. On dextrose agar the organisms are thicker and shorter, measuring 0.5 by 1 $\mu$. Involution forms are common, thick and swollen, measuring about 0.8 $\mu$ in diameter and 1.5 $\mu$ in length. Long forms with pointed ends may also occur, measuring 0.8 $\mu$ in width and 2.5 to 3.5 $\mu$ in length. The films from dextrose agar present great diversity of size and shape.

*Stained* easily with anilin dyes, best with dilute carbolfuchsin. Gram-negative.

*Motility* active in young cultures, lost in old cultures.

**Cultural Characters.**—*Cultivated* easily on the ordinary media.

*Plain Agar Plates.*—In twenty-four hours the colonies are round, regular, $\frac{1}{2}$ to 1 mm. in size. In forty-eight hours they are increased in size, measuring 1 to 3 mm., round, regular, with thin margins. Deep colonies pin-point. Under low power the superficial colonies show dense granular centers, thin margins, entire edges. Deep colonies under low power are round or oval, distinctly moruloid. In three days the colonies are rather thinner than the plain agar colonies of other spirilla. They measure about 3 mm. in size. The deep colonies are

Fig. 31.—*Vibrio Milleri* from dextrose agar culture twenty-four hours old, stained with gentian-violet.

mostly punctiform. In five to six days the superficial colonies are almost $\frac{1}{2}$ cm. in diameter. They show dense central heaped-up nuclei with thin margins. The deep colonies are usually round. In nine to twelve days the superficial colonies are $\frac{3}{4}$ to 1 cm. in size, showing thin margins and dense central nuclei, visible to the naked eye. In old plates the colonies are flattened and spreading with entire edges.

*Plain Agar Slant.*—In twenty-four hours a thick, raised, spreading, abundant growth. In forty-eight hours the growth is thicker and more abundant. In three to four days the growth is still more abundant, moist, rather spreading. In six to ten days the growth is thick, heavy, and whitish.

*Plain Agar Stab.*—In twenty-four hours an abundant growth occurs along the line of inoculation with a faint surface growth. In forty-eight hours the line growth is more abundant with more marked surface growth. In three to four days there is an abundant line and surface growth. In eight to ten days an abundant growth appears along the line of inoculation and a spreading growth on the surface.

*Dextrose Agar Plates.*—In twenty-four hours the superficial colonies are thin, translucent, $\frac{1}{4}$ to $\frac{1}{2}$ mm. in size. Deep colonies pin-point. No change in reaction of the agar. Under low power the superficial colonies are round, regular, thin, margins entire, peripheries light,

centers dark. The colonies may also show denser, central nuclei, or the centers of the colonies may be biconvex disks. Under low power the deep colonies are ovals or disks. In forty-eight hours the colonies are still small and thin, $\frac{1}{2}$ mm. in size. The plates remain acid. In four to eight days the colonies are still small and fine, $\frac{1}{2}$ to 1 mm. in size. Agar permanently acid.

*Dextrose Agar Slant.*—In twenty-four hours a moderate growth appears with the agar slightly acid. In forty-eight hours the growth is still moderate, and the reaction fairly faint. In four to five days considerable acidity develops with a fairly abundant growth. Acidity permanent.

*Gelatin Plates.*—The colonies appear in forty-eight hours as pin-point masses lying in drops of liquid gelatin. Under low power they are feathery with outlying strands, resembling the colonies of the anthrax bacillus. They are different from gelatin colonies of other vibrios. In three days the plates which show many colonies are entirely liquid. Other plates show many discrete colonies with zones of liquefaction about them. The centers of these colonies are about $\frac{1}{2}$ mm. in size. Under low power the colonies are feathery or woolly. In four days the zones of liquefaction are increased to 1 cm. in diameter. The colonies are now very dark and hazy. In ten days the colonies show dark centers, thin margins, with granular dots in the zones of liquid gelatin. These zones are about 1 to 2 cm. in width. Eventually all the plates become liquid.

*Gelatin Stab.*—In twenty-four hours a faint growth along line of inoculation with some cupping at the surface. In forty-eight hours increased growth along line and on the surface.

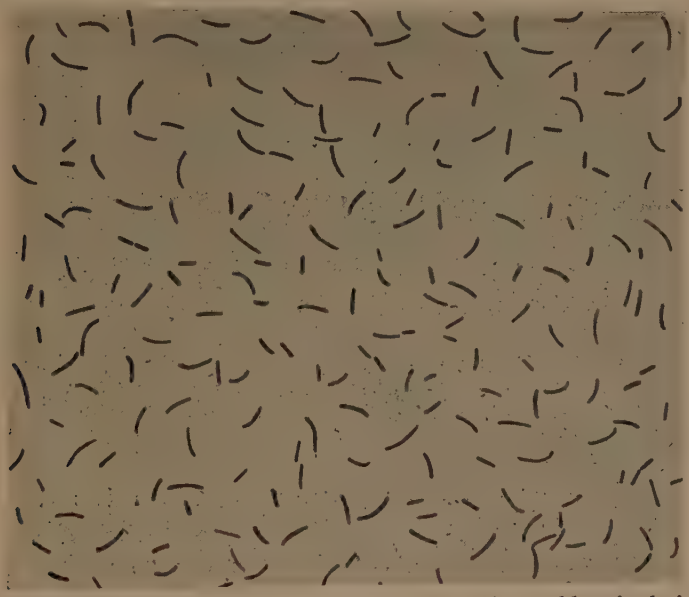


Fig. 32.—*Vibrio Milleri* from plain agar culture twenty-four hours old, stained with gentian-violet.

In three to five days there is considerable surface liquefaction. In ten days the gelatin is one-fourth liquefied. In older cultures the gelatin is entirely liquefied with a brownish sediment at the bottom.

*Blood-serum.*—In twenty-four hours moderate, raised, white, moist growth. In forty-eight hours the growth has a faint cream color. In three to five days the growth is somewhat increased and distinctly cream-colored. In ten days the growth is still more increased with some areas of softening. In sixteen days the areas of softening are increased to a distinct cupping, but there is no real liquefaction.

*Potato.*—No growth.

*Broth.*—In twenty-four hours turbidity, no scum, no sediment. In forty-eight hours the growth is very turbid with some sediment and slight ring growth. In three to five days the turbidity and sediment are more marked, but no scum develops. Old cultures the same.

*Peptone.*—In twenty-four hours turbidity, no sediment, no scum. In forty-eight hours turbidity and sediment increased, no scum. In three to five days the turbidity and sediment are more marked. No scum.

*Litmus Milk.*—Slight acidity in three to four days, increasing slowly.

*Fermentation Tubes.*

*Dextrose.*—In twenty-four hours turbidity in the bowl, no scum, no sediment. Arm faintly turbid. In two to three days turbidity in arm and bowl increased, no scum, slight sediment. Highly acid.

*Saccharose.*—Appearance the same. Highly acid.

*Lactose.*—Appearance the same. Slight acidity.

1. Miller: Deutsche med. Wchnschr., 1885, xi, 138.
2. Migula: p. 981.

### VIBRIO LINGUALIS (Migula)

First obtained by Weibel[1] as a vibrio from the surface of the tongue. Isolated from the organs of a mouse inoculated with material from the tongue. Named *Spirosoma linguale* (Weibel) by Migula.[2] Subsequently found by Bojardi[3] in plates made from the mouth secretions of normal individuals.

**Morphology.**—Curved rods about the size of the cholera vibrio and S forms. More frequently shorter or longer threads. These may be uniformly wavy or may show a series of angular bends. Occasionally the curves are so flat that the thread appears almost straight. Often button-shaped terminal enlargements are found, in some cultures being the predominant type. S forms often predominate in the cultures both in stained preparations and in hanging drops. Also round spherical forms occur singly or in pairs, giving an impression as if the comma or S forms had been reduced to buttons. These thickened organisms take the stain deeply and cannot be spores.

Unstained in twenty-four-hour broth cultures the organisms appear as fine, wavy, homogeneous rods with occasional refractile granules. They are isolated or form a network of organisms showing true branching. Old cultures show chiefly short thick forms. They look like a thin mycelium with dichotomous branching. The branches come off at points where refractile granules appear. No motility.

**Staining Reactions.**—Stained two to five minutes with Ziehl's fuchsin isolated or grouped organisms appear in which intensely stained areas are found. Short and compact organisms may have two granules and look like diplococci. Others are long and homogeneous with thick clubbed ends. Some have deeply stained rounded ends. Stained with Loeffler's alkaline methylene-blue the organisms appear as a row of blue bodies, small and punctiform, and looking as if separated by a protoplasmic net. Short forms may show two granules, long forms one larger terminal granule. Old cultures show homogeneously stained threads, short and thick forms and long Y-shaped organisms. With Neisser's stain a mass of organisms appear stained yellow with vesuvin. It contains a great number of granules, round, punctiform, often in pairs. These look like true diphtheria bacilli. Granules in rows, 2, 3, or 5. When many they are small, but when few they are larger. Club-shaped organisms appear with single granules on end. Gram-positive.

**Cultural Reactions.**—*Grows* both at 18° to 20° C. and at 40° to 42° C. Optimum temperature 37° C.

*Agar Plates.*—Colonies in twelve hours at 37°C., appear like thickets. Rarely the colonies show peripheral branches.

*Agar Slant.*—Dirty-white expansion at first moist, later dry. In old cultures very uneven with raised points. Canary-yellow in color.

*Agar Stab.*—Raised wide-spread expansion on the surface with fine wavy edges. This occasionally shows raised patches where apparently the colonies lie on top of each other. Moist, white, gradually turning yellow. Along line of inoculation a band with delicately jagged wavy edges. Colonies also develop on the outer edge of the agar and between it and the glass.

*Gelatin Plates.*—Characteristic colonies, dirty-white to naked eye, with a diameter of 0.3 to 0.5 mm. in a few days, and over 1 mm. in a week. Deep colonies under low power look like colonies of the anthrax bacillus. They consist of a mass of fine tangled filaments with projecting irregular processes. Superficial colonies show a light yellow-green shimmer, usually have round contours and thin edges with one or more, usually many, tangentially not radially arranged processes.

*Gelatin Stab.*—Raised round surface colony with depressed center and wavy dotted edges. Broken streak along line of inoculation giving off vertical side branches with club-shaped ends. Characteristic appearance like a lamp-brush. Gelatin gradually liquefied, the surface expansion sinking to the bottom. By the end of two months the gelatin liquefied to a depth of 2 cm. It remains clear below 20° C. At 20° C. it becomes turbid.

*Potato.*—Very slight development. Moist, barely visible expansion like the growth of the diphtheria bacillus on potato. Later, a limited dry linear expansion develops with regular edges.

*Broth.*—Growth like that of the anthrax bacillus. A flocculent coherent sediment at the

bottom of the tube and a light turbidity above this. The flocculent masses at the bottom are made up of thickly tangled filaments. In ten days cultures ring growth and delicate dirty-white superficial scum.

*Milk.*—Coherent citron-yellow ring growth and no coagulation.

No indol. No gas production.

Non-pathogenic.

1. Weibel: Centralbl. f. Bakteriol. [etc.], 1888, iv, 225.
2. Migula: p. 957.
3. Bojardi: Centralbl. f. Bakteriol. [etc.], 1904, xxxv, 129.

### VIBRIO WOLFII (Migula)

Isolated from the cervical secretions in a case of endometritis by Wolf.[1] Named *Microspira Wolfii* by Migula.[2]

**Morphology.**—Great variations in form. Usually definitely curved rods which often are half-circles. No spirals such as occur in the cholera vibrio. Also S forms, drumstick forms, and organisms with clubbed thickening at both ends.

**Staining Reactions.**—Stained easily with anilin dyes, anilin water, gentian-violet, carbolfuchsin, and Bismarck brown. Alkaline methylene-blue requires a long-time exposure and does not give as clear pictures as the other stains. Gram-negative. Many individuals fail to stain uniformly, but show terminal unstained areas.

*Motility.*—Active to-and-fro swarming movements in hanging drops kept at 37° C. twelve hours.

*Multiplication* apparently by transverse fission, especially in the S-shaped forms.

**Cultural Characters.**—*Gelatin Plates.*—Colonies appear within twenty-four hours as whitish-gray points in the depths, rapidly break through to the surface, and cause a spreading liquefaction. They are circular with sharp edges. They form funnel-shaped depressions in the center, in the middle of which appear small white clumps. By the second or third day the majority of the colonies are the size of lentils or pfennig pieces and frequently confluent. The gelatin has a slight milky odor.

Under low power (100 magnification) the smallest colonies have delicately toothed or jagged edges. They are white to yellow in color with a trace of gray and are clearly granular. As the colonies increase in size and begin to liquefy they become coarsely granular, especially toward the peripheries, while the centers remain finely granular and are marked off from the peripheries by a darker seam or line. Such colonies resemble colonies of the cholera vibrio. They are not always dark, but may be light colored as if made up of greenish glass beads or fragments.

*Gelatin Stab.*—Development along the entire line of inoculation. Liquefaction begins in twelve hours at a temperature of 25° C., starts at the surface and forms a funnel with an air-bubble in the upper wider portion. This is caused by the sinking of the superficial mass into the soft gelatin below. The larger part of the culture collects as white masses below this air-bubble on the line of inoculation. Here they lie as light yellow curly strands. Usually a spherical or club-shaped thickening forms at the lower part of the line. Liquefaction complete in four to six days at 25° C. and somewhat later at room temperature.

In *grape-sugar-gelatin* the growth is much the same, but slower with a slower liquefaction. The masses of bacteria are more yellow brown.

*Agar Stab.*—Growth slower than in gelatin with a smooth, glistening, wax-like surface expansion. In the depths the growth proceeds from above downward. In eight days it is 1 mm. wide with delicately toothed edges. Growth best at 37°. Slower at room temperature.

*Blood-serum.*—White sunken streak visible in twenty-four hours. Liquefaction is rapid, and in three to four days the entire medium is liquid.

*Milk.*—Clotted in forty-eight hours.

*Broth.*—Rapid development at 37° C. as a grayish-white scum with a touch of blue. This adheres to the sides of the test-tube and is frequently wrinkled. A little later the entire fluid has a slightly milky turbidity with clumps of coherent films precipitated to the bottom. The broth cultures also have a milky odor.

*Potato.*—Yellowish-white expansion, confluent at the edges, which become irregular and disappear. Growth at 37° C. fairly rapid.

The addition of concentrated sulphuric acid to a gelatin culture several days old or to a broth culture produces a rose-red ring. In a short time the fluid above takes on the same color which changes to a dark yellow in twelve hours.

Nitrites apparently not formed. The addition of sulphuric acid and potassium iodid starch paste does not produce a blue color. Pathogenic to mice by large doses.

1. Wolf: München. med. Wchnschr., 1893, lx, 692.
2. Migula: p. 1001.

### VIBRIO NASALIS (Migula)

Originally described by Weibel as a vibrio from nasal secretions. Named *Spirosoma nasale* (Weibel) by Migula.[2] Subsequently found in the mouth.

**Morphology.**—Fairly large organisms from the mucus at the back of the nasopharynx, about as thick as the anthrax bacillus and two to five times the breadth in thickness. Ends rounded. Great variations in degree of curvature from straight forms to half-circles. The shorter organisms are almost always straight. The organisms are in the mucus in groups. In stained preparations the individual organisms are surrounded by an unstained zone which does not appear like a capsule.

In pure cultures the organisms vary greatly with the media. In broth they appear as single rods of the same size as in the mucus. These forms show polar staining and look like diplococci. In gelatin and agar straight and curved rods appear and long chains of curved rods, which may appear as wavy and serpent-like threads or as closely-wound spirals. These spirals possess as many as 30 or more turns which are regularly disposed and so close to each other that they appear to touch. They are common on agar and on broth agar jelly, less frequent on gelatin. Often the different elements of a spiral differ in extent of curvature and a great variation in bending and curving results. Occasionally long threads appear which are so straight as not to reveal any curved individuals. Finally involution forms are very common, like those in the cholera vibrio, clubbed thickenings, irregular swellings, large spheres, etc. No spores. Non-motile.

**Staining Reactions.**—The vibrios stain easily with aniline dyes, especially with gentian-violet. Decolorized by Gram's method. For the polar staining organisms good preparations are made by overstaining the films in gentian-violet and washing quickly in dilute alcohol (1–3 in water).

The vibrios are destroyed by drying on a coverslip.

**Cultural Characters.**—*Gelatin Plates.*—Colonies grow slowly, appearing as whitish points in three days. In five days they attain a diameter of 0.3 mm. and later about twice this, after which they stop growing. Under low magnification (80 times) each colony appears as a round disk with sharp edges, yellow-brown, finely granular by transmitted light. In old colonies a concentric dark ring appears near the edge. *No liquefaction.*

*Gelatin Stab.*—Delicate, white, veil-like or cobwebby streak develops in a few days along the entire line of inoculation. This appears to the naked eye as if made up of minute granules (colonies). Occasionally the line of inoculation below is represented by small isolated colonies like those of *Streptococcus pyogenes.* No surface development or spreading from the point of puncture.

*Agar Stab.*—Growth like that in gelatin, but more rapid and abundant, especially at 37° C. The streak along the line of inoculation is thicker, opaque, less regular, showing thick turbidities at various points. Small, flat, dirty-white viscid layer at point of puncture.

*Broth Agar Jelly.*—Broth containing enough agar to have a jelly-like consistency. In this medium a diffuse turbidity spreads from the line of inoculation and if several deep punctures are made at the same time the entire mass of medium is penetrated by the growth.

*Broth.*—Turbidity in a few hours at 36° C., reaching its maximum on second to third day. The vibrios diminish in number after this time.

*Potato.*—No growth at room or body temperature.

No growth in *starch solutions, hay infusions,* or *solutions* of *gum* with mineral salts.

No odor.

1. Weibel: Centralbl. f. Bakteriol. [etc.], 1887, ii, 465.
2. Migula: p. 955.

### VIBRIO TONSILLARIS Stephens and Smith

Originally found by Klein in the throat of a diphtheria patient, but described first and named by Stephens and Smith who isolated it several times in cultures from the throat along with the diphtheria bacillus.

**Morphology.**—Pleomorphic vibrios, appearing in young cultures on serum agar as delicately curved rods and larger spiral forms the size of Finkler-Prior's vibrio. On ordinary agar it appears as comma forms, half circles, and spirals. In gelatin shorter and thicker, and only slightly curved or pointed organisms arranged in pairs with the thicker ends approximated.

Stained easily by basic anilin dyes.

Gram-negative.

*Motility* active and progressive. Flagella stained by van Ermengem's method. They are 1 to 2 in number, terminal, on one or both ends. Each flagellum has 4 to 5 spiral turns and is longer than the vibrio itself. They may also be stained by Pitfield's method.

**Cultural Characters.**—*Agar Plates.*—Small transparent disks with opaque centers.

*Agar Slant.*—Rapid development at 37° C. as an opaque expansion. This gradually thickens and becomes yellow in the middle. The edge is irregular and made up of isolated colonies.

*Agar Stab.*—Fine delicate growth along the line of inoculation with a thin leaf-like surface expansion.

*Serum Agar Slant.*—Growth like that on plain agar.

*Gelatin Slant.*—Dry transparent flat growth with finely granular processes running out from jagged edges. Individual colonies after eight or more days appear as clearly marked zones of brighter or darker color. The gelatin in the neighborhood is colored whitish. The growth resembles that of the diphtheria bacillus. No liquefaction.

*Gelatin Stab.*—Development as a thread of small transparent grayish drops along the line of inoculation with a transparent disk on the surface. No liquefaction or gas formation.

*Gelatin Plates.*—Colonies 1 to 1.5 mm. wide, appearing as disks with opaque centers and brighter and darker transparent zones. These may be surrounded by cloudy zones with wavy markings.

*Milk* not clotted.

*Potato.*—Moist, greenish yellow, moderately thick growth after several days.

*Peptone.*—Uniform turbidity in twenty-four hours at 37° C. No scum.

*Broth.*—Uniform turbidity in twenty-four hours at 37° C. No scum.

Indol reaction negative.

Non-pathogenic.

Stephens and Smith: Centralbl. f. Bakteriol. [etc.], 1896, xix, 929.

### VIBRIO SURATI (Laub and Paton)

Isolated from a case of vegetative endocarditis by Laub and Paton from the vegetations and from the patient's blood. Named *Spirillum surati* by them.

**Morphology.**—In the tissues slightly curved rods, comma and S-shaped forms, arranged as spirilla with eight to ten turns. Somewhat smaller curved rods, commas and spirilla in cultures. Single organisms about 3 to 4 $\mu$ in length. The long spirilla measure as much as 23.5 $\mu$. Turns vary from 3 or 4 to as many as 16. The ends of the long forms are noticeably pointed. Organisms usually thin and delicate.

*Motility* active. *Stains* with ordinary anilin dyes and best with carbolfuchsin. Gram-negative.

*Cultivated* on ordinary media.

*Ascites Glucose Agar.*—Most favorable medium. At the end of twenty-four hours minute semitransparent dew-like colonies with a very slight whitish sediment in the water of condensation. Colonies larger in forty-eight hours. Old colonies $\frac{1}{2}$ to $\frac{3}{4}$ cm. in diameter, round, with a serrated or irregular border. Margins clean-cut. Raised slightly, dirty white. With transmitted light the centers appear thick and brownish, peripheries white and more translucent. Under low power the margins appear slightly raised with a suggestion of radiating lines from periphery to center. The sediment in the water of condensation becomes very sticky and rolls up into a little ball-like clump which can be separated on shaking. Growth on surface becomes quite tenacious and adheres to the medium.

*Glucose Agar.*—Colonies smaller than those on ascites glucose agar and develop more slowly.

*Plain Agar.*—Growth slower, more uncertain and never as profuse.

*Blood Agar.*—Colonies appear in twenty-four to forty-eight hours, minute, whitish, glistening, the color of the medium shining through. At the end of a week the colonies are 3 to 4 mm. in diameter. They look like ascites agar colonies.

*Loeffler's Blood-serum.*—Growth slow, not profuse.

*Plain Broth.*—Very slight diffuse cloudiness in twenty-four hours with a slight sticky sediment. More marked cloudiness in forty-eight hours, sediment increased in amount, whitish in color. It is quite sticky and forms a long tree-like whitish upshoot when the tubes are twirled.

*Glucose Broth.*—No growth in twenty-four hours. At the end of forty-eight hours, slight cloudiness in open arm of the fermentation tube, none in closed arm. No gas. At the end of a week increased turbidity and slight whitish sediment on the sides of the tube.

*Lactose Broth.*—Growth like that in glucose broth. No gas.

No fermentation of *inulin*.

No growth in *litmus milk*.

No liquefaction of *gelatin*.

No *indol*.

Grows well under anaërobic conditions.

Non-pathogenic to white rats, guinea-pigs, and rabbits.

Laub and Paton: Arch. Int. Med., 1913, xii, 259.

### VIBRIO METCHNIKOVI Gamaléia

Obtained by Gamaléia[1] from the intestinal contents and blood in an epidemic of gastro-enteritis in fowls. Apparently the same organism was found by Kutscher[2] in water. Extensively investigated by Pfeiffer and Nocht.[3] Present description from an old Johns Hopkins strain originally obtained from Europe.

**Morphology.**—On plain agar twenty-four hours old the organisms appear as slightly curved vibrios, measuring about 0.4 $\mu$ in width and 1.5 to 2 $\mu$ in length. Longer forms 3.5 $\mu$ in length and long threads 10 to 12 $\mu$ in length are not uncommon. Occasionally short forms, only about 1 $\mu$ long, are seen. Rarely typical spirals are formed by the juxtaposition of several vibrios. On dextrose agar twenty-four hours old involution forms are common, ovals measuring 0.6 to 0.8 $\mu$ by 1 to 1.5 $\mu$, round bodies about 1.5 $\mu$ in diameter, long irregular rods, 1 $\mu$ in width and 6 to 7 $\mu$ in length. Rarely large spirals are visible in the cultures.

Fig. 33.—*Vibrio Metchnikovi* from culture on plain agar twenty-four hours old, stained with gentian-violet.

*Motility* very active from single long polar flagella. The long forms have a serpentine movement. After three to four days growth the motility is usually lost.

*Stains* well with the usual anilin dyes. Gram-negative.

*Cultivated* easily on ordinary media, best at 37° C.

**Cultural Characters.**—*Plain Agar Plates.*—In twenty-four hours the surface colonies are round, regular, ½ to 1 mm. in size. Deep colonies pin-point. Under low power the superficial colonies are round, regular, with central nuclei, margins thin, edges entire. Deep colonies, ovals or disks. In forty-eight hours the surface colonies are 1 to 2 mm. in size, round, and elevated, while the deep colonies are round, oval or biconvex disks to the naked eye. In six to eight days the superficial colonies are 2 to 3 mm. in size, thick and opaque with dense centers. Deep colonies when older are distinct biconvex disks.

*Plain Agar Slant.*—In twenty-four hours a thick, abundant, moist, slightly yellowish growth develops. In forty-eight hours this is very abundant, and moist. In four to six days the surface growth is very heavy and brownish. In nine to ten days the growth is still more abundant, distinctly brown in color.

*Plain Agar Stab.*—In twenty-four hours an abundant line growth develops, with some surface growth. In forty-eight hours both line and surface growth are more marked. In four days the growth is increased, especially on the surface. In nine to ten days the surface growth extends to the sides of the tube and is distinctly brownish in color.

*Dextrose Agar Plates.*—In twenty-four hours the plates are acid, the superficial colonies ½ to 1½ mm. in size, round and regular. Under low power they are round, regular, uniform, with small central nuclei, margins thin, edges entire. Deep colonies pin-point, under low power chiefly biconvex disks or ovals. In forty-eight hours the colonies are about the same size, but in three days they are slightly larger, some colonies being 2 mm. in diameter. In ten to twelve days the plates are all alkaline. The colonies are now ½ to ¾ cm. in diameter, thin, flat, centers dark, margins entire. The colonies may be acid, with the rest of the agar alkaline. In older plates the colonies may still show acid centers.

*Dextrose Agar Slant.*—In twenty-four hours fairly abundant, thick, raised growth. Agar slightly acid. In forty-eight hours the growth is very abundant. In four to five days it is heavy and yellow, with the agar alkaline. In nine to ten days the growth is very heavy, the agar deep blue.

*Gelatin Plates.*—The colonies grow slowly, none appearing in twenty-four or forty-eight hours, but in three days thickly seeded plates may be entirely liquefied. Other plates show small colonies, ¼ mm. in size, lying in areas of liquid gelatin about 2 mm. in diameter. Under low power the colonies are round with regular edges, somewhat straggling, but not like "proteus" colonies. In four to five days the colonies are still small and round, ½ mm. in size, dense, with areas of liquefaction, ¾ cm. about them. Under low power the colonies have a very woolly or cottony appearance, dense centers with outlying feathery margins. The colonies float about in the liquid gelatin. In ten days the plates are almost entirely liquefied. Colonies which are left show dense centers, granular periphery, sitting in zones of liquid gelatin. Gelatin colonies darker in color than cholera colonies.

Fig. 34.—*Vibrio Metchnikovi* from culture on dextrose agar twenty-four hours old, stained with gentian-violet.

*Gelatin Stab.*—In twenty-four hours a faint line growth with considerable surface cupping. In forty-eight hours considerable growth along the line of inoculation with marked surface liquefaction following the cupping. In four to five days the liquefaction is increased. In nine to ten days the gelatin is almost completely liquefied with a heavy sediment at the base, especially in the line of growth. The gelatin at the top is quite clear. In twelve to fifteen days the gelatin may be entirely liquefied, with a sediment at the bottom of the tube.

*Blood-serum.*—In twenty-four hours an abundant growth, particularly in the water of condensation. The serum is dark and sunken along the surface inoculation. In forty-eight hours considerable liquefaction of the serum occurs. In four days this is increased. In ten to fifteen days the serum is soft, partially liquefied, and collects in the bottom of the tube.

*Potato.*—No growth.

*Broth.*—In twenty-four hours turbidity and sediment, but no scum. In forty-eight hours the turbidity and sediment are increased and a faint scum appears. In four to five days the scum is heavy, the turbidity and sediment increased. In nine to ten days turbidity, scum, and sediment are more pronounced.

*Peptone.*—In twenty-four hours faint turbidity and sediment, but no scum. In three to four days the turbidity is increased with a faint sediment, but no scum. In nine to ten days the turbidity and sediment are much more pronounced, with a heavy scum.

*Litmus Milk.*—Faint acidity and decolorization of the litmus in four to five days. This increases slowly and the proteins of the milk are gradually thickened. Coagulation appears late, usually after two to three weeks. Eventually the coagulum is quite firm, with the expression of considerable serum.

**Fermentation Tubes.**—*Dextrose.*—In twenty-four hours turbidity and precipitate in arm and bowl, no scum, and no line of demarcation. In forty-eight hours the turbidity in bowl and arm are increased, with slight sediment. No scum. Highly acid.

*Saccharose.*—Appearance the same. Highly acid.

*Lactose.*—Appearance the same. Moderate acidity.

Cholera-red reaction positive.

**Pathogenic Action.**—In the natural disease in fowls the birds are somnolent with ruffled plumage. The illness is somewhat longer than fowl cholera, lasting forty-eight hours or more. It is regarded by Gamaléia as a gastro-enteritis, while fowl cholera is more of an enteritis. At autopsy the principal lesion is a hyperemia of the intestinal tract. The spleen is not enlarged, but small and pale. The organism is present in the intestinal contents. It is also present in the blood of young chicks, but not in the blood of adult animals which is not infectious. In the natural disease in pigeons the vibrios are present in the blood in pure culture.

*Pigeons* are very susceptible to experimental inoculation, a few drops of a culture inoculated under the skin or in the muscles killing them in eight to twelve hours. According to Pfeiffer and Nocht the injection of small amounts left on the point of a needle into a wound of the skin produces a fatal septicemia. This helps to distinguish the organism from the cholera vibrio, large doses of which have no effect on pigeons. At autopsy the intestines are filled with seropurulent material, the spleen is exsanguinated. The organisms appear in the heart's blood in enormous quantities. The vibrios do not produce disease in pigeons when given by mouth even in large quantities.

*Fowls* are usually less sensitive than pigeons to subcutaneous or intramuscular inoculation. They succumb quickly when the organisms are introduced by mouth.

*Rabbits* and *ground squirrels* are refractory, but are killed by heavy inoculations. *Guinea-pigs* are very sensitive to any type of dosage. Subcutaneous injection of minute traces in a pocket of the skin produces a fatal septicemia. Intraperitoneal introduction of small quantities, 1/100 of an oesa, produces a rapidly fatal peritonitis, with the vibrios in the blood. The organism is thus ten times as virulent for guinea-pigs as the cholera vibrio, which requires 1/10 of an oesa to produce fatal infection. Guinea-pigs also succumb to infection by the stomach even if the gastric juice is not neutralized. The animals die in twenty-four hours. At autopsy the intestines are filled with fluid containing exfoliated epithelium and the spleen is anemic. The vibrios are present in large numbers in the intestinal contents, in the blood, and in the organs.

Animals may be immunized to *Vibrio Metchnikovi* by the introduction of small doses. These immunized animals give a Pfeiffer reaction with this organism, but not with the cholera vibrio.

1. Gamaléia: Ann. de. l'Inst. Pasteur, 1888, ii, 482.
2. Kutscher: Ztschr. f. Hyg., 1895, xix, 461.
3. Pfeiffer and Nocht: Ztschr. f. Hyg., 1889, vii, 259.

### Vibrio Smithii (Migula)

Described originally by Smith[1] from the large intestines of swine. Found in abundance in the soft necrotic material at the base of small ulcers. Named *Microspira Smithii* by Migula.[2]

**Morphology.**—Actively motile vibrios from broth cultures, somewhat larger than the cholera vibrio. The organisms are usually comma-shaped, but spirals may occur with 2 to 10 turns. The flagella are easily stained by Loeffler's method and are polar, resembling those of the cholera vibrio.

**Cultural Characters.**—*Cultivated* without difficulty on the ordinary laboratory media. Markedly aërobic.

*Gelatin Plates.*—Colonies appear in thirty-eight to forty-eight hours as small, round, finely granular disks of brownish color. Superficial colonies, small round expansions, irregular in size. In roll-tubes the deep colonies have a diameter of 0.3 to 0.5 mm. After several days they show concentric layers or zones. In several weeks they have a characteristic appearance

not unlike the flat surface of a sawed tree trunk, and show many feebly marked concentric rings. On the periphery of many of the deep colonies short, thickly intertwined processes develop which give the colonies the appearance of raspberries. Superficial colonies attain a diameter of 3 to 5 mm. when the gelatin is prevented from drying. They retain their round flat shape and become slightly yellow.

*Agar Plates.*—Deep colonies 0.5 to 0.7 mm. in diameter. Superficial colonies, flat round disks, grayish in color and smooth in texture. They may grow to a diameter of 5 mm.

In *neutral broth* with $\frac{1}{4}$ to 1 per cent. peptone the organisms grow well. After several days at 36° C. the fluid is very turbid. The organisms grow better in broth which is not neutralized. The reaction becomes alkaline. No growth in acid broth. The tendency to scum formation is marked. The scum is whitish, very thin and friable. It consists of very long spiral forms, some of them moving inertly.

*Fermentation tubes* containing peptone broth and 2 per cent. grape sugar. No growth in closed arm. The organisms have no fermentative action on sugar.

*Milk* not changed microscopically.

*Potato.*—Thin yellowish expansion after several days in the thermostat.

Not pathogenic to pigeons by intravenous inoculation, to guinea-pigs by intraperitoneal inoculation, or to swine by feeding.

1. Smith: Centralbl. f. Bakteriol. [etc.], 1891, x, 177.
2. Migula: p. 1006.

### VIBRIO SUBTILISSIMUS (Migula)

Obtained by Kutscher[1] from manure and swine dung (Spirillum No. 1). Regarded by Kutscher as probably identical with the spirillum found by Smith[2] in swine dung. Described separately and named *Spirillum subtilissimum* by Migula.[3]

**Morphology.**—The smallest of the spirilla cultivated up to that time. Not thicker than the delicate spirilla often present in the human intestine. Pointed at the ends. Appear usually as S forms and short spirals with 3 to 4 curves or turns.

*Motility* active. Flagella not stained.

**Cultural Characters.**—*Gelatin Plates.*—Colonies characteristic. They show 3 zones. The center is formed by a compact light or dark glistening nucleus. This is surrounded by curly, finely granular masses of bacteria, the edges of which are frequently lost in a wreath of anastomosing strands. Slow liquefaction.

*Gelatin Stab.*—Development along the entire line of inoculation as a whitish thread or line from which cloudy tufts extend into the gelatin in all directions. Gradually the gelatin is liquefied and evaporated from above downward and a funnel-shaped air bubble forms, slowly sinking in the medium.

*Agar Plates.*—Deep colonies small, colorless or light yellow, whetstone in shape, with small lateral protuberances. When superficial colonies develop the agar seems to be covered with small transparent dew drops. Under low power these appear as delicate, finely granular expansions with sharp edges.

*Agar Slant.*—Delicate growth.

*Agar Stab.*—Growth only in upper part of line of inoculation. Surface growth not characteristic.

*Blood-serum.*—Non-characteristic growth.

*Potato.*—No growth.

*Milk.*—No growth.

*Agar Meat Broth.*—Slight turbidity, no scum.

Non-pathogenic.

Optimum temperature 37° C.

1. Kutscher: Ztschr. f. Hyg., 1895, xx, 46.
2. Smith: Centralbl. f. Bakteriol. [etc.], 1895, xvi, 324.
3. Migula: System der Bakterien. 1900, ii, 1020.

### VIBRIO SUIS

Described originally by Kutscher[1] from dung manure as Vibrio No. 2. Put in the spirilla and named *Spirillum coprophilum* by Migula.[2]

**Morphology.**—Twice the length and thickness of the cholera vibrios. In old fluid cultures long, actively motile chains appear.

*Motility* active, due to six fine slightly curved polar flagella which are often bound together in 1 to 2 bunches.

**Cultural Characters.**—*Grows* well at temperatures from 22° to 37° C.

*Agar Slant.*—Abundant coherent non-characteristic growth.

*Agar Plates.*—Deep colonies greenish-yellow, finely granular round or whetstone in shape. The superficial colonies form delicate expansions yellow in the middle with an outer colorless zone of finely toothed edges.

*Agar Stab.*—Growth along entire line of inoculation. Surface growth not characteristic.

*Gelatin Plates.*—Colonies at first shimmering lightly, finely granular, greenish to greenish-yellow with slightly wavy or sharp edges. As they develop they become deep yellow, lose their outer markings, and become homogeneous in the centers. On the surface they form delicate, transparent, finely mottled expansions with jagged edges. These expansions thicken and microscopically resemble *Bacillus coli.* Microscopically they are differentiated by their darker color and their lack of markings. No liquefaction.

*Gelatin Stab.*—Abundant whitish development along the line of inoculation, forming isolated colonies at the lower portion. On the surface a whitish expansion, with a lacquer-like lustre and deeply jagged edges extending to the walls of the tube.

*Blood-serum.*—Non-characteristic expansion.

*Potato.*—Thin grayish-white dry layer covering the cut surface.

*Milk.*—Development, but no change in reaction.

*Agar Meat Broth.*—Rapidly developing uniform turbidity with superficial scum.

Non-pathogenic.

1. Kutscher: Ztschr. f. Hyg., 1895, xx, 46.
2. Migula: System der Bakterien. 1900, ii, 1019.

### VIBRIO HYOS

Reported first from dung manure by Kutscher[1] as Vibrio No. 3. Transferred to the spirilla and named *Spirillum mobile* by Migula.[2]

**Morphology.**—Smaller slightly than *Vibrio serpens* with rounded ends. Long chains rare. S forms common. Frequently the cell protoplasm is very granular and tends to form vacuoles.

*Motility* active due to bunches of delicate, slightly curved terminal flagella, often 8 in number.

**Cultural Characters.**—*Growth* at temperatures from 22° to 37° C. Organisms at 37° C. motionless, or almost motionless. Most active motility in cultures below 30° C.

*Agar Plates.*—Deep colonies whetstone in shape, greenish-yellow to brown in color, with lateral protuberances of various sizes. The superficial colonies appear first as delicate, irregular, finely mottled expansions. Later they become much thicker, brown in color in the center, and are traversed by wavy anastomosing furrows. The edges are very jagged and show tongue-like projections.

*Agar Stab.*—Growth only in upper part of line of inoculation. Surface growth not characteristic.

*Agar Slant.*—Isolated colonies only with rich inoculation.

*Gelatin Plates.*—Young colonies like the "bone-cell" colonies of the mouse septicemia bacillus. Older colonies show larger compact nuclei, turn yellow, and become finely granular. The gelatin is slowly liquefied.

*Gelatin Stab.*—Development along entire line of inoculation. More abundant above than below. It first appears as a veil-like turbidity from which delicate feathery processes extend into the clear gelatin. Gradually the gelatin is liquefied and evaporated from above downward, and eventually a funnel-shaped canal with an air-bubble is formed.

*Blood Serum.*—Non-characteristic growth.

*Potato.*—Whitish dry growth slightly raised from the surface.

*Milk.*—No growth.

*Agar Meat Broth.*—Turbidity uniform and abundant. Superficial scum.

Non-pathogenic.

1. Kutscher: Ztschr. f. Hyg., 1895, xx, 46.
2. Migula: System der Bakterien. 1900, ii, 1020.

### VIBRIO INTERMEDIUS (Migula)

Noted originally by Kutscher[1] as Group V No. 9 of the organisms, resembling the cholera vibrios. Found in the dejections and intestinal tract of swine. Named *Microspira intermedia* by Migula.[2]

*Motility* active from single polar flagellum.

**Cultural Characters.**—*Agar* growth on plates, slant and stab like that of the cholera vibrio, except that it is inhibited at 27° C. and sparse at 37.5° C.

*Gelatin plates* thickly seeded are like plates of the cholera vibrio, except that the zones of

liquefaction are larger. In thinly seeded plates the zones of liquefaction extend under the edges of the colonies and are bladder-like. Occasionally the colonies show radiation and break up into individual granules.

In *gelatin stabs* a stocking-like liquefaction develops in several days, the liquefied gelatin being turbid. After more extensive liquefaction the gelatin clears with a reddish-white precipitate of bacterial growth. On the surface of the gelatin a delicate coherent scum develops.

*Blood-serum* is rapidly peptonized at 22° C. by a whitish growth. At 37.5° C. the serum is covered by a thin expansion, but is not peptonized.

No growth on *potato*.

In *broth* and *peptone* rapid development of a uniform turbidity with a delicate scum at 22° C. Little development at 37.5° C.

*Milk* not changed. Growth abundant.

Nitroso-indol reaction positive in cultures grown at 22° C. and transferred to 37.5° C. Also in old strains in cultures kept at 37.5° C.

**Pathogenic Action.**—Mice injected subcutaneously develop a local edema and slough which gradually heals. Pigeons refractory to intramuscular inoculation.

Guinea-pigs injected intraperitoneally show a condition like that with the cholera vibrio, actively motile vibrios being present in the peritoneal exudate.

1. Kutscher: l. c., p. 481.
2. Migula: l. c., p. 967.

### VIBRIO GROSSUS (Migula)

Described from dung by Kutscher[1] as Vibrio No. 1. Named by Migula[2] *Microspira grossa.*

**Morphology.**—Like the cholera vibrio.

*Motility.*—Like the cholera vibrio. Single polar flagellum with several spiral turns.

Good growth at temperatures from 22° to 37° C.

**Cultural Characters.**—*Agar Plates.*—Deep colonies yellow, whetstone in form, frequently showing lateral protuberances. Superficial colonies appear as delicate, colorless homogeneous expansions, somewhat irregular in size.

*Agar Slant.*—Abundant coherent non-characteristic growth.

*Agar Stab.*—Growth only in upper portions. Superficial expansion not characteristic.

*Gelatin Plates.*—Deep colonies coarsely granular, greenish, with uneven edges, looking like young cholera colonies. Superficial colonies show sometimes a greenish central mass with many anastomosing fissures, surrounded by an outer, delicate, homogeneous colorless zone with fringed edges. No liquefaction.

*Gelatin Stab.*—Delicate veil-like development along line of inoculation. Thick expansion on the surface looking like frosted glass and extending to the walls of the tube. No liquefaction.

*Potato.*—No growth.

*Milk.*—Development without change of reaction.

*Blood-serum.*—Non-characteristic growth.

*Agar Meat Broth.*—Abundant uniform turbidity with superficial scum.

Non-pathogenic.

1. Kutscher: Ztschr. f. Hyg., 1895, xx, 46.
2. Migula: System der Bakterien. 1900, ii, 1012.

### VIBRIO FETUS Smith and Taylor

First isolated by Smith[1] in infectious abortion in cattle and described by Smith and Taylor.[2] Subsequently shown by Smith[3] to be always associated with cases showing the same clinical complex.

**Morphology.**—Fine, wavy, or sinuous lines of various lengths from fetal fluids and cultures. The smallest forms appear as minute curved S-shaped lines, the longest may stretch nearly across the field of the microscope. Diameter of spiral small. In some cultures the organisms are drawn out to almost a straight line. In dried and stained preparations the spiral becomes a shallow sinuous line without segmentations. Stained in alkaline methylene-blue, width not over 0.2 to 0.3 $\mu$, shortest form 1.5 to 2.0 $\mu$ long. Common size in fetal fluids consists of 2 complete turns and measures 4 to 5 $\mu$ in length. Average diameter of spiral about 0.5 $\mu$.

The organisms *stains* well in alkaline methylene-blue when staining is prolonged, preferably over night. Stains much more deeply in diluted anilin gentian-violet. It is thicker when stained by Moore's[4] modification of Loeffler's flagella stain. The flagella are single

and attached to one or both poles. Unipolar flagella are more numerous than bipolar flagella. Occasionally longer spirilla are found with several short flagella in addition to the terminal ones. The flagella are very fine hair-like processes in large undulations or waves, of uniform width throughout.

*Motility.*—The short forms with ½ to 2 windings are very active, shooting with lightning rapidity in straight lines in all directions. In a few cases the organisms revolve about the longitudinal axis. The longer forms move slowly or are quiescent.

*Granules* appear on or in the spirilla, especially in old cultures, and are probably the product of degeneration. The long spirilla may contain many granules, but the shorter forms may also contain some. In the latter case they are terminal. In the long-forms they are arranged along the filament at fairly regular intervals. They stain much more deeply than the rest of the spirillum and suggest the Neisser granules of the diphtheria bacillus.

**Cultural Characters.**—The earliest cultures were obtained in agar slants containing a small quantity of water of condensation or added broth. To this a small bit of tissue from the fetus, about ⅛ gram, or a small amount of the stomach or intestinal contents was added. The tubes were sealed with sealing-wax. After three to four days two very narrow grayish-white opaque lines of growth, less than 1 mm. broad, extend upward from beneath the surface of the water of condensation where the agar slant abuts the glass. Later an exceedingly thin veil-like layer extends from each of these two lines into the capillary space between the agar and the glass, often meeting behind and forming a complete, barely visible layer with upper margins. It varies in height and is undulating or jagged, rarely more than 4 cm. above the bottom of the tube. The water of condensation may be very feebly clouded. Surface growth very rare. If horse blood or mammalian blood is added to the water of condensation a layer of growth appears on the surface of the sedimented corpuscles. This grows heavier with the number of transfers. Surface growths appear after months of cultivation, either as grayish films or as isolated, roundish, smooth glistening colonies 1 to 2 mm. in diameter. After a time the cultures grow more vigorously and multiplication takes place on agar slants without blood. Strains can now be maintained on plain agar in sealed tubes. A viscid white sediment appears in the water of condensation. This may be drawn out into threads. It may point to a kind of mucoid degeneration. This is suggested by unstained sheaths about the spirilla. In old saprophytized strains growth may be obtained by mixing fluid agar with the spirillum and allowing it to set. Growth in sealed tubes is gradual, appearing after two to three days as a fine white line around the circumference of the surface of the agar and slowly growing down between the agar and the glass as a fine film. Condensation water gathers, becomes viscid and growth spreads over the surface of the agar. At the end of about a week a zone of individual colonies forms in the agar beneath the surface. Such colonies are small, opaque and yellow. The zone may appear directly beneath the surface or 2 to 5 mm. below. In unsealed tubes there is no water of condensation and no surface growth. The subsurface zone appears heavier than in sealed cultures.

*Agar Stab.*—Growth slow in sealed cultures, appearing on the second day along the line of puncture. The final growth is 5 mm. to 3 cm. in length. When a lateral puncture is made a fine white film spreads between agar and glass. Surface growth at the point of puncture. Water of condensation becomes viscid. In unsealed cultures no surface growth.

Feeble growth on *coagulated horse serum.* No liquefaction.

Growth in *slant agar* containing defibrinated blood. No growth in *gelatin.*

Old strains grow in *beef peptone broth* in sealed and unsealed tubes, multiplication being more vigorous if a few drops of defibrinated blood be added. The medium becomes faintly cloudy and a slight viscid sediment forms. If the tubes are slightly inclined, a filmy stringy deposit may settle upon the sides and a viscid ring at the surface, but no pellicle.

No growth on *potato.*

Organisms survived in sealed original cultures at room temperature a variable time, from thirty-one to one hundred and thirty-nine days. Subcultures on blood agar varied similarly, one dead in two and one-half weeks, another alive after nine weeks. Vitality reduced at 5° to 6° C.

No indol.

*Dextrose, lactose,* and *saccharose* broth in fermentation tubes showed uniform cloudiness in the bulbs and a slight deposit. Branch clear. No gas. Reaction after five days neutral or slightly acid.

*Destroyed* by drying three hours at room temperature, but survives two hours, drying. Withstands a temperature of 55° C. for five minutes, but not for ten minutes.

**Pathogenic Action.**—The injection of pure cultures into the fetal membranes produced disease in 2 out of 4 instances. Agglutination reactions the same in 21 fetal strains and in 1 calf strain.

1. Smith: J. Exper. M., 1918, xxviii, 701.
2. Smith and Taylor: J. Exper. M., 1919, xxx, 299.
3. Smith: J. Exper. M., 1919, xxx, 313.
4. Moore: Wilder Quarter Century Book, Ithaca. 1893, p. 339.

### Vibrio cardii Klein

Isolated by Klein from the heart mussel, *Cardium edule.*

**Morphology.**—Characteristic comma-shaped vibrios with many S forms and short spirals. They are slightly smaller than the cholera vibrio when taken from colonies on Conradi-Drigalski medium. In other culture media like agar, gelatin, broth, etc., the vibrios in the first few days are very small, slightly curved, and extremely motile. They have 1 to 2 short flagella on one end. Later they assume the typical comma forms and the S forms like those on Conradi-Drigalski medium. On ordinary agar, gelatin and blood-serum plano-convex, biconvex and spherical forms are common just as with the cholera vibrio in rice water stools.

**Cultural Characters.**—*Cultivated* easily on the usual media. The colonies on *Conradi-Drigalski medium* are blue, roundish, conical, raised in the center and flat on the edges. When isolated they attain a diameter of several millimeters in two to three days. A funnel-shaped liquefaction is produced in *gelatin stabs* like that from the cholera vibrio. The liquefied gelatin is not very turbid. *Coagulated blood-serum* is slowly liquefied to a browny, syrupy mass. *Litmus milk* acidified and coagulated after several days. Development good in *peptone broth* containing salt. A partial scum or film is formed on the surface in several days. This consists microscopically of an interlacing mass of wavy and spiral threads. No *nitroso-indol* reaction.

Injected subcuteously in guinea-pigs *Vibrio cardii* produces only localized tumors. Intraperitoneal inoculation of 1 oesa of twenty-four- to forty-eight-hour agar cultures kills guinea-pigs in twenty hours with purulent peritonitis.

Klein: Centralbl. f. Bakteriol. [etc.], 1 Abt., 1905, xxxviii, 173.

### Klein's non-pathogenic mussel vibrio

This form was isolated several times by Klein from mussels, *Mytilus edulis*, from sewage and from sea water in which the mussels were placed. It shows actively motile, comma-shaped vibrios, S forms and short spirals. The organisms have 1 to 2 cilia on one end.

*Cultivated* easily on ordinary media. Small, round, sky-blue colonies on *Conradi-Drigalski medium.* Transparent expansion on *gelatin* which is not liquefied. Slow, scanty growth in *peptone* solution containing salt. No surface scum even after several days.

No *nitroso-indol* reaction.

Non-pathogenic.

Klein: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1905, xxxviii, 173.

### Bergman's vibrio from Keratomalacia of codlings

Found by Bergman in an infectious disease of codlings (*Gadus morrhua* L) on the south coast of Sweden. In the natural disease the cornea of one or both eyes first becomes gray and opaque, then is perforated and the entire eye destroyed. From the infected material vibrios were isolated which closely resembled *Vibrio anguillarum* found by Bergman in a tumor-producing disease of eels.

**Morphology.**—Slightly curved vibrios 0.5 $\mu$ thick and $\frac{1}{2}$ $\mu$ long. In three-day agar cultures the unstained vibrios are 0.6 to 0.8 $\mu$ thick, 1.5 to 1.8 $\mu$ long, often in chains of not more than 4 individuals. Actively motile. Gram-negative. *Grows* best at room temperature, also at 40° C. and at 37° C. Facultative anaërobe.

**Cultural Characters.**—Dry, gray expansion on *agar.* Uniform turbidity in *broth* and *peptone*, with a superficial film in three to four days. Broth cultures have an odor like that in sewers. *Gelatin* liquefied. *Milk* acidified and coagulated. Acid, no gas from glucose, saccharose, lactose and maltose. No $H_2S$ formation. No *nitroso-indol* reaction. No proteinchrome formation in 3 per cent. peptone solution. No reduction of indoxylsulphonic acid (Indigosulfonsäure natron).

**Pathogenic Action.**—Direct injection of pure cultures into the cornea of codlings, eels and roaches, produces ocular changes like those observed in the natural disease. The organisms are also virulent to eels, crabs and roaches by subcutaneous injection. Not pathogenic for the carp, or for rabbits and guinea-pigs. Agglutinins may be produced in rabbits by intravenous injection of living cultures. They vary in strength from 1 to 1000 to 1 to 10,000.

Bergman: Centralbl. f. Bakteriol, [etc.], 1. Abt., 1912, lxii, 200.

———: Ber. a. d. K. Bayr. Biolog. Versuchsstat. München, 1909, ii.

### VIBRIO ANGUILLARUM Bergman

Isolated by Bergman from an infectious tumor-producing disease of eels. Larger than the vibrios from codlings, measuring 2 by 8 $\mu$ in agar cultures. Cultural reactions about the same except that lactose is not attacked and milk is not acidified or coagulated. According to Bergman there is apparently a group of vibrios pathogenic for fishes, including this organism from eels, the vibrios from codlings and a vibrio found by him in inflammation about the teeth in pike.

Bergman: Ber. a. d. K. Bayr. Biolog. Versuchstat. München, 1909, ii.

### VIBRIO TYROGENUS (Deneke)

Described originally as *Spirillum tyrogenum* by Deneke from cheese. Present description from a culture from the American Museum.

**Morphology.**—On plain agar twenty-four hours old, small, almost straight comma-shaped organisms, about 0.4 $\mu$ in diameter and 1.5 to 2 $\mu$ in length. Longer forms measuring 2.8 to 3.5 $\mu$ are also present and occasionally short spirals. On dextrose agar the organisms are longer and thinner, measuring about 0.4 $\mu$ in width and 1.5 to 3.5 $\mu$ in length. Many of these are definitely curved.

*Stained* with the usual anilin dyes; best with carbolfuchsin. Gram-negative.

*Motility* very active from single polar flagella.

**Cultural Characters.**—*Cultivated* easily on the usual media. Growth is slow, especially at room temperature.

*Plain Agar Plates.*—Colonies grow slowly, appearing in forty-eight hours round, regular, measuring $\frac{1}{2}$ to $1\frac{1}{2}$ mm. in size. Deep colonies pin-point. Under low power the superficial colonies have dense granular centers with thin margins and entire edges. Often the nucleus is a deep biconvex colony. Under low power the deep colonies are biconvex disks which may be distinctly corrugated. In three days the superficial colonies are 2 mm. in size. The deep colonies still punctiform. In five days the superficial colonies are 3 mm. in diameter, the deep, slightly increased in size, mostly round, but occasionally ovals. In eight to twelve days the colonies are still small, 3 mm. in size, with darker centers and thin peripheries.

*Plain Agar Slant.*—In twenty-four hours a thin, moist, abundant, spreading growth. In forty-eight hours the growth is increased and is yellowish at the bottom. In three to four days it is somewhat more abundant. In cultures ten to twelve days old the growth is quite heavy and yellow-brown in color.

*Plain Agar Stab.*—In twenty-four hours a faint line growth with a slight surface growth. In forty-eight hours the line growth is still faint, but there is considerable surface growth. In three to five days the growth is more abundant along the line and on the surface. In ten to twelve days the growth is increased, especially on the surface, and in older cultures it extends to the wall of the tube.

*Dextrose Agar Plates.*—The surface colonies appear in twenty-four hours, $\frac{1}{2}$ to 1 mm. in size, round and regular. Deep colonies are pin-point. Under low power the superficial colonies are round, regular, with dark centers, thin margins, and entire edges. They may also show folded centers like *Vibrio proteus*. The deep colonies are oval with folds or are biconvex disks. The plates are all acid in twenty-four hours. In three days the plates are still acid, the colonies are much larger, measuring $\frac{1}{4}$ cm. in diameter, showing dark centers, round or bizarre jagged edges. In ten to twelve days the agar is alkaline and greenish blue. The colonies measure $\frac{1}{2}$ to $\frac{3}{4}$ cm., showing dark centers, thin peripheries and irregular margins. Under low power the edges of the colonies are irregular, but entire. In old plates the colonies may still show traces of acid while the agar about them is alkaline.

*Dextrose Agar Slant.*—In twenty-four hours a thick, raised, yellow growth. No acid. In forty-eight hours the growth is very thick, raised, yellowish with some acidity at the top of the agar. In three to five days the growth is increased, red in the center and yellowish on the margins. The agar is highly acid. Acidity permanent or may give way to an alkaline reaction.

*Gelatin Plates.*—Growth very slow, colonies appearing in three days as very fine dots. Under low power they are round, regular with entire edges. In four days the colonies are somewhat increased in size without liquefaction. The colonies remain small and in ten days measure only $\frac{1}{2}$ to 1 mm. in diameter. Under low power they are round, regular and uniform. No liquefaction.

*Gelatin Stab.*—Growth slow, appearing only in three days as a faint line and surface growth without liquefaction. In five days the line and surface growth are slightly brownish and increased in extent. In ten days the surface growth is quite brown in color. The gelatin is not liquefied.

*Blood-serum.*—In twenty-four hours a dry, yellowish, slightly elevated growth. In two to three days the growth is quite yellow, raised, without liquefaction of the serum. In cultures eight to ten days old the growth is abundant and yellowish in color. The serum is not liquefied.

*Broth.*—In twenty-four hours some turbidity, no sediment, no scum. In forty-eight hours turbidity and sediment are increased, with a slight ring growth, but no scum. In three to five days the turbidity is more marked. In cultures ten to twelve days old the turbidity is heavy, the sediment marked, and a viscid growth appears in the upper portion of the broth.

*Peptone.*—Very faint turbidity in twenty-four hours, no scum, and no sediment. The turbidity remains faint and in five to eight days there is little or no sediment and no scum.

*Litmus Milk.*—Gradual production of an alkaline reaction which may become marked in six to eight days. No coagulation.

*Potato.*—No growth.

*Fermentation tubes:*

*Dextrose.*—In twenty-four hours turbidity in bowl, no scum, no sediment, arm clear. In forty-eight hours the turbidity in the bowl is marked. Slight turbidity in arm. Line of demarcation fairly distinct. No scum. No acid.

*Saccharose.*—Appearance the same. No acid.

*Lactose.*—Appearance the same. No acid.

No *nitroso-indol* reaction.

Non-pathogenic.

Deneke: Deutsche med. Wchnschr., 1885, xi, 33.

### VIBRIO AQUATILIS Günther

Isolated by Günther[1] from the water of the River Spree in Berlin. The same organism found by Kiessling[2] in the water at Altona.

**Morphology.**—In young gelatin cultures the organisms are moderately thick rods with rounded or pointed ends. They are about 0.6 $\mu$ in breadth and 1.5 to 2 $\mu$ in length. In agar cultures they are longer and thinner with pointed ends, often forming half circles. Involution forms are common at high temperatures. They consist of thick, long coiled plump threads swollen in the middle, half-moon forms, and spheres. In agar cultures one to two days old at 37° C. thick spirals occur in tangled masses like balls of thread. These show flexion and torsion and may have a length of 80 $\mu$.

**Staining Reactions.**—Young forms stain well. In old cultures the dead organisms stain with difficulty, as do the involution forms, which show refractile polar bodies.

*Motility* active like that of the cholera vibrio. The organisms have a single cilia at one end only.

Grows well at 21° to 22° C. and at 37° C. Highly aërobic.

**Cultural Characters.**—*Gelatin Plates.*—Circular colonies with smooth edges as if cut out in a circle. They are brownish in color and show extremely fine granulations. Later they lose their sharply cut contours.

*Gelatin Stab.*—Growth almost exclusively on the surface, where it forms a basin-shaped depression of liquid gelatin which gradually deepens. No growth along the line of inoculation beneath the surface.

*Agar Plate.*—Colonies like those on gelatin both at 15° to 20° C. and at 37° C.

*Agar Slant.*—Good growth on the surface at 37° C.

*Potato.*—No growth.

*Broth.*—No growth at 37° C. in alkaline or neutral meat extract salt broth. After some weeks slight turbidity at 21° to 22° C.

*Nitroso-indol* reaction negative in broth cultures. Well-developed agar cultures washed off with dilute NaCl solution give reaction for both indol and nitrites.

Non-pathogenic to white mice, field mice, pigeons, guinea-pigs, and rabbits.

1. Günther: Deutsche med. Wchnschr., 1892, xviii, 1124.
2. Kiessling: Arb. a. d. K. Gsndhtsamte, 1893, viii, 430.

### VIBRIO MARINUS (Russell)

Found in sea-water and sea mud by Russell at Naples, and described as *Spirillum marinum.*

**Morphology.**—Small bent or slightly curved bacillus, usually in pairs. Several elements may be joined together, producing a characteristic spirillum.

*Motility.*—Rapid progressive and rotatory movements.

**Staining Reactions.**—Stains lightly with Loeffler's methylene-blue and deeply with fuchsin.

*Grows* only at low temperatures. No growth at 37° C. Grows better on ordinary media than on media made from sea water.

**Cultural Characters.**—*Gelatin Plates.*—Round, small, radially striped granular colonies which liquefy the gelatin. In the liquid gelatin floating flocculent particles appear.

*Gelatin Stab.*—Rapid growth with a thin translucent pellicle on the surface. Liquefaction with turbidity of the liquid gelatin.

*Sea-water Gelatin Stab.*—More restricted growth with a thick pellicle on the surface of a shallow liquid depression which is filled with a turbid fluid.

*Agar.*—Abundant moist, liquid, whitish expansion resembling pus in appearance. The growth collects at the bottom of the tube and becomes watery.

*Potato.*—Reddish-brown, sharply circumscribed growth in twenty-four hours. This increases in size and covers the whole potato, remaining soft and superficial. The potato gradually becomes dark grayish green.

*Sea-water Broth.*—Abundant growth with heavy turbidity, an abundant white deposit, and a smooth white pellicle.

*Plain Broth.*—Moderate turbidity.

Russell: Ztschr. f. Hyg., 1891, xi, 165.

### VIBRIO BEROLINENSIS Neisser

Isolated by Neisser[1] from the filtered water of the Stralauer water supply. Named *Microspira berolinensis* (Neisser), Migula by Migula.[2]

**Morphology.**—Small curved rods, usually somewhat smaller than the cholera vibrios, but varying in size. Frequently 2 vibrios lie end to end. Rarely threads and spirals appear in old agar cultures. Involution forms common, staining with difficulty. The curve of the organism is about like that of the cholera vibrio. No spores.

*Motility* active due to a long single terminal flagellum, spirally wound.

**Staining Reactions.**—Gram-negative.

**Cultural Reactions.**—Growth at 20° to 22° C. and at 37° C. on the usual media. More active growth than that of the cholera vibrio except on gelatin, where it is poorer.

*Gelatin Plates.*—Colonies visible under low magnification in twenty-four hours as small and round with sharp edges. Vivible macroscopically in forty-eight hours. They develop in all depths of the gelatin, are homogeneous or finely granular, pale, transparent, colorless, often looking like fat-drops. They frequently look bluish when contrasted with the surrounding medium. Surface colonies spread, are transparent, film-like, with round colonies as central nuclei. No formation of funnels of liquefaction. The edges remain sharply defined. The twenty-four-hour colonies are smaller than those of the cholera vibrio.

Liquefaction of the gelatin develops, but much more slowly than with Koch's organism.

*Gelatin Stab.*—Development along upper part of line of inoculation with an air bubble like that of the vibrio of Asiatic cholera. Liquefaction proceeds slowly, the air bubble remaining small and the funnel-shaped liquefaction developing only at the upper part. Whitish-gray growth along line of inoculation below.

*Agar.*—Fine grayish-yellow, slightly raised, transparent, moist glistening growth in twenty-four hours at room temperature, somewhat thicker and less transparent at 37° C. Growth on glycerin agar like that on ordinary agar.

*Agar colonies* like those of the cholera vibrio, but less transparent.

*Potato.*—On natural slightly acid potato the growth is yellowish and moist at all temperatures from 17° to 37° C. in twenty-four hours. Later it becomes viscid and yellowish brown. On potato mashed in 1 per cent. soda solution the growth is moist and yellowish white. It is also yellowish white on potato washed with 1 per cent. acetic acid, but somewhat yellowish brown on potato boiled in 3 per cent. salt solution.

*Broth.*—In alkaline broth marked turbidity in twenty-four hours at 22° and at 37° C., with a white, firm, superficial scum in forty-eight hours.

*Pancreas Broth.*—Abundant growth.

*Peptone* (1 per cent. peptone, 1 per cent. NaCl).—Turbidity in forty-eight hours.

*Milk.*—No change in reaction. No coagulation.

*Grape-sugar* in fermentation tubes. Turbidity in bowl and arm and no fermentation.

Destroyed at 60° C. in a few moments.

Dies out in three days at a temperature of 10° C.

*Indol* reaction positive.

*Nitroso-indol* reaction positive.

Non-pathogenic to mice, rabbits, and pigeons.

1. Neisser: Arch. f. Hyg., 1893, xix, 194.
2. Migula: System der Bakterien. 1900, ii, 974.

### VIBRIO DANUBICUS Heider

Described by Heider from the water of the Danube canal at Vienna.

**Morphology.**—Similar to the cholera vibrio except that more S forms appear with moderate curving and rounded ends. In old broth cultures long spiral forms appear with shallow curves.

*Motility* active. Flagella stained by Loeffler's method, without acid or alkali added to the mordant, appearing as cilia with two to three curves one and one-half times the length of the vibrio. In young agar cultures eight to ten hours old each organism has a single flagellum attached to the end. Two or more flagella at one end of the organism not observed.

**Cultural Reactions.**—*Gelatin Plates.*—Colonies appear on thickly seeded plates in sixteen hours, very small, round, very pale, and homogeneous. They develop rapidly and in twenty-two to twenty-four hours at 20° to 22° C. such plates appear turbid by transmitted light as if from large particles of dust. The surface is rough and finely pitted. Under low power the colonies are round, bright or light gray, with a shade of yellow, surrounded by a lighter zone with finely toothed edges and finely granular surface. Many of these colonies resemble colonies of the cholera vibrio. In addition, a few flat spreading colonies are found, irregular round expansions with wavy or coarse processes projecting from the edges. These are almost colorless and finely and regularly marked.

On thinly seeded plates twenty-two hours old the superficial colonies are almost entirely these last described. Deep colonies are round with smoother edges and slightly granular surface. Nearly all show a bright sheen, the first indication of liquefaction. After thirty hours the thickly seeded plates are liquefied and the others show slight hollows with whitish points at the bottom. Such colonies appear to the naked eye like cholera colonies. Microscopically they are round, coarsely granular disks, light grayish-brown in color, with smooth edges which are formed by regularly interspersed granules. Those which liquefy rapidly appear as round colonies with warty edges and coarsely indented surfaces, surrounded by a light zone. Such colonies are microscopically much like cholera colonies. These colonies soon disintegrate, crumbling masses breaking off from the edges and lying along the walls of the zone of liquefaction. They now no longer resemble cholera colonies. The typical colonies form circular depressions with beautiful wreath-like radiations, in the midst of which a regular granular mass of light brownish-yellow color is seen. In plates forty-eight hours old the colonies are 1.5 to 2 mm. in diameter, in three days 3.5 to 4 mm., in thin places 5 mm. Liquefaction begins in the middle and for a time the center of the colony appears as a small saucer filled with turbid fluid, surrounded by a delicate zone of the original growth.

While in early stages the colonies resemble those of the cholera vibrio, in late stages they are much more like those of *Vibrio Metchnikovi*. They may be differentiated by the slower liquefaction.

*Gelatin Stab.*—Growth like that of the cholera vibrio in rapidity of liquefaction. There is, however, a more marked growth in the first few days and a more uniform turbidity in the liquefied gelatin.

*Broth.*—Rapid development of turbidity at both room and thermostat temperature. Gradually a superficial scum develops containing long spiral forms.

*Agar Slant.*—Abundant whitish expansion like that of the cholera vibrio.

*Milk.*—Clotted in forty-eight to seventy-two hours.

*Potato.*—Slow development of a yellowish-brown expansion in the thermostat. At room temperature after about a week only a slightly moist glistening fleck.

*Litmus broth* powerfully reduced. The blue color disappears in twelve to sixteen hours at 37° C. almost completely.

*Nitroso-indol* reaction positive in five to six hours in peptone solution, either with 1 per cent. peptone and ½ per cent. NaCl or 2 per cent. peptone and ½ per cent. NaCl. Sometimes the reaction is not given. This is due not to failure of nitrite formation, but to failure of indol.

Odor like that of cholera vibrio cultures.

*Bujwid's Iodoform Test.*—Gelatin inoculated with *Vibrio danubicus* and treated with iodoform steam is softened after four to five days. No liquefaction of gelatin by the cholera vibrio under the same conditions in ten to fifteen days.

*Virulent* to pigeons by intramuscular injection at times, not so regularly as *Vibrio Metchnikovi*. *Virulent* to guinea-pigs by intraperitoneal inoculation of larger doses than are required for the cholera vibrio, by alimentary adminstration by Koch's method, and by direct injection into the duodenum. *Virulent* to mice by intraperitoneal inoculation. *Virulent* also to guinea-pigs by direct administration through the trachea. Subcutaneous injections fatal to mice. Differentiated from the cholera vibrio and from *Vibrio Metchnikovi* chiefly by animal inoculation.

Heider: Centralbl. f. Bakteriol. [etc.], 1893, xiv, 341.

### VIBRIO SCHUYLKILLIENSIS Abbott

Obtained by Abbott from the water of the Schuylkill River.

**Morphology.**—In plain agar twenty-four hours old the organisms appear as well-marked vibrios, measuring about 0.4 $\mu$ in width and 1 to 2 $\mu$ in length. Some organisms are longer, measuring 2.5 to 3.5 $\mu$. True spirals are occasionally present 5 to 7 $\mu$ in length. On dextrose agar the elements are much enlarged, measuring usually about 0.7 to 0.8 $\mu$ in width and 3 to 4 $\mu$ in length. Long thin spirals also appear with plump rods, pear-shaped bodies, and large oval forms with pointed ends.

*Stained* easily with anilin dyes. Gram-negative.

*Motility* very active in young cultures, lost in old cultures.

Single polar flagella.

*Cultivated* aërobically on the usual media. Optimum temperature 37° C.

**Cultural Reactions.**—*Plain Agar Plates.*—In twenty-four hours the superficial colonies are round, regular, uniform, $\frac{1}{2}$ to $1\frac{1}{2}$ mm. in size. Deep colonies pin-point. Under low power the superficial colonies are round, regular, with central nuclei, thin margins, edges entire.

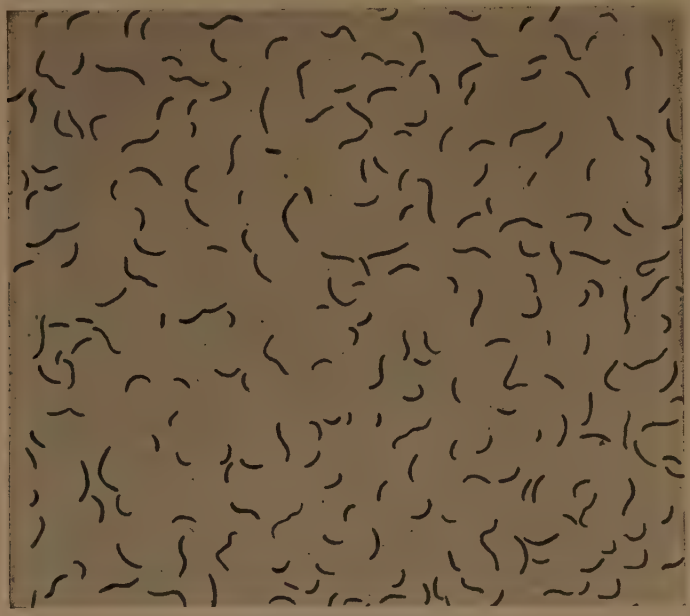


Fig. 35.—*Vibrio Schuylkilliensis* from culture on plain agar twenty-four hours old, stained with gentian-violet.

The central nuclei may be deep colonies. Under low power the deep colonies are biconvex disks. In forty-eight hours the superficial colonies are 1 to 3 mm. in size. The deep colonies are round, oval, or biconvex disks to the naked eye. In six to eight days the superficial colonies are $\frac{1}{2}$ cm. in size, the deep colonies still biconvex disks. In older plates the surface colonies are thick and opaque with thin margins, while the deep colonies are well-developed biconvex disks.

*Plain Agar Slant.*—In twenty-four hours a thick, abundant, smooth, moist, slightly spreading growth. In forty-eight hours it is thicker, but still moist. In four to five days it spreads over the agar and in old cultures the medium is covered by a whitish expansion.

*Plain Agar Stab.*—In twenty-four hours a moderate growth along line of inoculation with considerable surface growth. In forty-eight hours the line growth is heavy with a spreading surface growth. In four to six days the surface growth is yellow and in old cultures it spreads to the walls of the tube as a brownish expansion.

*Dextrose Agar Plates.*—In twenty-four hours the superficial colonies measure $\frac{1}{2}$ to $1\frac{1}{2}$ mm. in size. Under low power they are round, regular, edges entire, margins thin, slightly granular. Deep colonies pin-point, under low power ovals or disks. Plates acid. In forty-eight hours the isolated colonies are flat, 1 to 2 mm. in size, with dark centers. Under low power they show dark granular centers, thin margins, and entire edges. Plates still acid. In three to five days the colonies measure $\frac{1}{2}$ cm. in size with thin peripheries and entire edges. In

older plates, ten to twelve days, the colonies are quite large, often 1 cm. in diameter, and may have a pinkish color from the acidity. The agar usually is permanently acid.

*Dextrose Agar Slant.*—In twenty-four hours a fairly abundant growth with some acidity. In forty-eight hours the growth is heavy and the acidity increased. In eight to ten days the agar is nearly covered with a flat expansion. Acidity usually permanent.

*Gelatin Plates.*—Colonies appear in forty-eight hours small and white, measuring $\frac{1}{2}$ mm. in size, lying in cups of liquid gelatin. Under low power they show feathery edges and are woolly, just like *Vibrio proteus*. In three to four days the colonies are very hazy and sit in zones of liquid gelatin about $\frac{3}{4}$ to 1 cm. in diameter. In six to ten days the gelatin is entirely liquefied.

*Gelatin Stab.*—In twenty-four hours a faint line growth with considerable surface cupping. In forty-eight hours the growth is abundant along the line and the cupping increased. In four days marked cone-shaped surface liquefaction appears. In eight to nine days there is a marked surface liquefaction. This is progressive, and in about two weeks the gelatin is entirely liquefied.

*Blood-serum.*—In twenty-four hours a brown growth, sunken along the line of inoculation. In forty-eight hours a marked surface liquefaction appears. In four days the serum

Fig. 36.—*Vibrio Schuylkilliensis* from dextrose agar culture twenty-four hours old showing involution forms, stained with gentian-violet.

is about one-fourth liquefied. The liquefaction is progressive, and in nine to ten days the entire mass is softened and semifluid.

*Broth.*—In twenty-four hours turbidity and sediment, no scum. In forty-eight hours turbidity increased, a slight sediment forms, but no scum. In old cultures, eight to ten days, the turbidity is heavy, the precipitate marked, but some formation is lacking.

*Peptone.*—Turbidity and sediment. No scum.

*Litmus Milk.*—Slight acidity in three to four days, increased slowly. No coagulation even on heating.

*Potato.*—No growth.

**Fermentation Tubes.**—*Dextrose.*—Turbidity in bowl and arm. No scum. Slight sediment Highly acid.

*Saccharose.*—Appearance the same. Highly acid.

*Lactose.*—Appearance the same. Highly acid.

*Nitrates* reduced to nitrites.

*Indol* positive.

Abbott: J. Exper. M., 1896, i, 419.

### VIBRIO BONHOFFII (Migula)

Originally described as a Vibrio by Bonhoff[1] from the water at Stolp, Pommerania. Named *Microspira Bonhoffii* by Migula.[2]

**Morphology.**—About the size and shape of the cholera vibrio in stained preparations from twenty-four-agar cultures, but less curved. Somewhat larger than many cholera vibrios, for instance *Vibrio Massauah*. Frequently S forms occur, the elements in them being smaller than the single individual cells. On the distal ends these S forms run out into long processes. Also smaller rods attached end to end are seen, thicker in the middle and quite pointed at the free ends. Also much longer, clearly bent forms, especially in young cultures, thicker at one end and tapering toward the other. These are often in S forms. They may be twice as long as the typical commas, but not much thicker. They take stains irregularly, showing several irregular light areas in their contents. They are evidently old organisms. The typical forms also show light colorless places inside the cells. These, however, are shown only by staining a short time in strong dyes, as carbolfuchsin. They lie always in the middle of the organism, are circular, light, and glistening. They resemble the analogous structures seen in the cholera vibrio. They are not spores, and forms showing them are as easily destroyed by heat as the usual organisms. In old broth cultures long spirals with 4 to 6 curves or turns appear in the surface scums.

*Motility* active, circular or rotating. This is due to a spiral flagellum attached to one end, stained by Loeffler's method.

**Staining Reactions.**—Stains by the basic anilin dyes, best by exposure a quarter of an hour in the cold. Gentian-violet and dahlia give the best pictures. Also good results with Ziehl's solution diluted ½ with water. Stains badly with methylene-blue, but well with Bismarck brown, methyl-green, and acid eosin. Iodin solutions turn the vibrios brown.

Gram-negative. Easily decolorized by weak acids. Not decolorized by acetic acid in water (1 : 10,000).

**Cultural Characters.**—Growth best on moist media, either slightly alkaline or free from any traces of free acid, at 37° C. Less growth at 18° to 25° C., still less at 15° C. and none at 10° C. No growth above 40° C.

*Agar Plates.*—At 37° C. in twenty-four hours the superficial colonies are grayish blue, pin head to pea size, and seem to consist of a delicate scum or film spreading over the surface of the agar. The majority show more or less projecting yellow-gray centers. Under low power this central point increases to a fine circle from day to day. Such colonies resemble *Vibrio Deneke* colonies on gelatin, but are darker. The superficial scum is surrounded by a lighter zone about 2 mm. wide. The edges are circular and sharply separated from the surface of the medium. This scum develops in all directions till growth is stopped by the drying of the medium.

*Agar Slant.*—Growth rapid. In four hours at 37° C. a moist, glistening, grayish-blue expansion develops with a delicate grayish-white, easily broken scum on the water of condensation and a thick, gray, viscid mass below. No color produced in the agar. The expansion in twenty-four hours is about 1 mm. thick, slightly more immediately above the water of condensation.

*Glycerin Agar* (4 per cent.).—More abundant growth layer, thicker, with clear markings like ridges on the surface. The medium takes on a light green color.

*Grape-sugar Agar* (1½ per cent.).—Growth like that on ordinary agar.

Abundant development in alkaline *broth* (peptone 1 per cent., NaCl 0.5 per cent.) in twenty-four hours as a fine grayish surface scum. This is whiter, not so glistening and delicate as with the cholera vibrio. Slight turbidity increasing in forty-eight hours with a faint sediment. The film is thicker and adheres to the walls of the tube. The addition of glycerin or grape sugar to the broth renders the growth a little more active. In *sugar broth* a bladder-like expansion on the surface, due possibly to a fermentation of the sugar and an accumulation of $CO_2$. In broth cultures two to four weeks old the sediment is much increased, the turbidity is greater and the superficial scum broken with individual portions swimming on the surface. No red color produced by the addition of sulphuric or hydrochloric acid to the broth.

*Gelatin Plates.*—Small colonies, barely the size of pin heads, visible only under the microscope in twenty-four hours (Leitz 3 Ocular 1). In forty-eight hours the colonies are visible to the naked eye as small pin-points, grayish in color. Under low power these are circular, silver gray, with sharp edges and individual, moderately glistening particles within. The colonies are larger on the third day, appearing as small, gray white points to the naked eye. Under low power these colonies are surrounded by light narrow halos. They consist of small particles which are darker and slightly grayish-yellow. They are still circular, no longer glistening. On the fourth day the colonies show definite walls, consisting toward the center of bright glistening particles, smooth toward the periphery. On the sixth day the colonies are twice the size of those on the fourth day. The superficial colonies have a pale grayish-yellow color and moist glitter. They are about the size of half-peas and cover the

still acid gelatin with a relatively thick scum. Under the microscope these colonies appear as flat masses of brown-yellow spheres balled together, with irregular edges, slightly granular. The colonies just below the surface are much the same. In the next few days the grayish-yellow, moist, glistening film spreads over the gelatin in all directions and forms an expansion the size of a 20-pfennig piece. This film is thicker than the superficial colonies of *Bacterium coli*, uniformly smooth and even without traces of markings.

*Gelatin Stab.*—Growth begins on the surface in three days and appears as a film like that seen in old gelatin colonies. A slight development occurs in the upper part of the line of inoculation, none below. Occasionally the gelatin turns green.

*Gelatin Slant.*—Silver-gray, glistening, transparent expansion, somewhat dry and reaching its maximum in four days.

Gelatin 10 per cent. in all cases and made slightly alkaline with soda solution.

*Potato.*—Round, yellow-brown drops in twenty-four hours at 37° C. No special increase in size, but become dry and darker. At 22° C. the growth is fine and brown and the whole potato turns dark.

*Beef Serum* (coagulated).—In twenty-four hours at 37° C. a fine transparent silver-white glass-like film with a fine scum on the water of condensation.

*Milk.*—No development in the first fourteen days. Then a collection of yellow fat-drops on the surface, the white milk loses its color, becomes dirty yellowish, then gray-yellow, and finally bright amber-colored, almost transparent. A whitish sediment collects on the bottom which may consist of lact-albumin and salts of phosphoric acid. The casein in this altered milk may be precipitated by the addition of a few drops of acetic acid to it after dilution. It is not peptonized, but dissolved by the strong alkaline which the vibrio produces in milk.

Growth only aërobic.

*Nitroso-indol* reaction in 1 per cent. peptone solution.

Destroyed at 55° C. in twenty minutes.

Slightly pathogenic to guinea-pigs.

1. Bonhoff: Arch. f. Hyg., 1893, xix, 252.
2. Migula: p. 1008.

### VIBRIO PORTUENSIS (Migula)

Reported by Jorge[1] as a new water vibrio from the water supply of the town of Porto in Portugal. Named *Microspira portuensis* by Migula.[2]

**Morphology.**—When originally isolated a slightly curved rod or more frequently a straight bacillus. After continuous transfers the organism appeared as a typical vibrio, uniform and regular in appearance. In broth usually short coccus-like organisms, mostly as diplococci, rarely longer or curved forms. Longer on potato, but often slightly curved rods. On agar, long curved rods and comma-shaped forms, but more frequently the straight rods. Longer and thinner than Koch's vibrio on agar. In more recent cultures the curved forms are more frequent and spirals also occur. Half-moon-shaped forms with pointed ends on albumin. On gelatin, longer and almost entirely curved rods; also many irregular forms like the letters C, W, S and O; comma forms and curling forms, together with spirals with 2 to 3 turns. Two or three organisms may often be attached end to end. This highly developed type of morphology usually appears on gelatin three to four weeks old, but may develop after a few days. The organisms may be involution forms. Occasionally swollen ends were to be noted like the oviform bodies in Koch's vibrio.

Originally the vibrio had a low vitality and tended to be converted into granules like strings of pearls. These were embedded in an amorphous mass and gave the aspect of cocci in a zoöglœa mass. Stains with the usual anilin dyes.

*Motility* very active, often radiating about an axis. Flagella stained by Loeffler's method, one or two at one end of the vibrio. Organisms with a flagellum at both ends or with three flagella rare. Flagella five to six times as long as the vibrio, spirals with several narrow curves. These look not unlike corkscrews.

**Cultural Characters.**—When first isolated, growth in the usual media very slow and only at room temperature. Not above 30° C. After several passages the organism grew more rapidly and abundantly and very well at 37° C.

*Agar Slant.*—Whitish growth along the line of inoculation, in twenty-four hours raised in the middle with irregular edges. This does not reach the wall of the tube. It resembles the growth of the cholera vibrio.

*Gelatin Plates.*—Colonies appear in two to three days as white points. Under the microscope, round finely granular disks with entire edges. These are closely surrounded by a concentric, light, less granular zone. Deep colonies do not grow more than pin-head in size. The superficial broaden out, become the color of mother-of-pearl, transparent, and develop irregular jagged edges. The lower part of the colony grows upward and shows radial furrows which run into the finely granular peripheral ring. The whole looks like a cross-section of a

glacial mountain especially when the gelatin begins to dry. While these colonies remind one of the colonies of the typhoid bacillus the latter are much larger and their edges less wavy and cut-out.

*Gelatin Streak.*—Irregular milk-white expansion with wavy edges.

*Gelatin Stab.*—Poor growth. No liquefaction. Barely visible fine granules along line of inoculation and on the surface a delicate whitish layer with irregular edges, seldom extending more than ½ the radius of the tube. It looks like the head of a pin.

*Coagulated albumin* slowly dissolved.

*Potato.*—At temperatures below 37° C. after three days a barely visible, moist, glistening fleck like the growth of the typhoid bacillus. The culture grows poorly and takes on a light-brown color.

*Broth.*—Uniformly, but slightly turbid in forty-eight hours without surface scum. After some time, a sediment.

*Peptone.*—No scum.

*Milk.*—No coagulation.

No special diastatic properties except the ability to dissolve coagulated albumin. No gaseous fermentation of *glucose* or *lactose*, but sugar solutions become acid. Produces *indol* and reduces nitrates. No decolorization of media containing fuchsin such as occurs with the typhoid bacillus.

Not acutely virulent for guinea-pigs by intraperitoneal inoculation. Some of the animals die of cachexia.

1. Jorge: Centralbl. f. Bakteriol. [etc.], 1896, xix, 277.
2. Migula: p. 1007.

### VIBRIO PERCOLANS Mudd and Warren

Isolated by Mudd[1] and Warren[2] from hay infusion.

**Morphology.**—Short, actively motile, comma-shaped to straight rods with rounded ends occurring singly or in short chains. Motility progressive with rotation on the long axis. Motion reversible. There is typically one polar flagellum though frequently two are present. The flagella are rarely bipolar; in length they range from 3 to 7 $\mu$. With the fuchsin encre stain of Nicolle and Morax they are readily demonstrated. Length of organism ranges from 0.5 to 2.5 $\mu$, the large majority being 1.5 to 1.8 $\mu$ long. Thickness 0.3 to 0.4 $\mu$. In young cultures (eighteen to twenty hours) filamentous forms are frequently found, some of which are chains of organisms, while in some subdivision is made out with difficulty or not at all. The filaments are typically long slender spirals; in smears they often appear straight or with only slight curvature. No spores.

*Staining Reactions.*—Stains with ordinary dyes. Gram-negative. Protoplasm appears homogeneous except for one or two fine granules in an occasional organism. Brightly shimmering coarse granulations appear by dark-field illumination in old non-motile organisms and in organisms in hay-infusion too acid for optimum growth. Involution forms in old (ten to twenty days) acid hay infusion cultures, similar to those of the cholera vibrio; large globoid forms which stain faintly, spoon-shaped and clubbed organisms, and forms which show a banded appearance with carbolfuchsin. Involution forms not so common as with the cholera organism.

**Cultural Reactions.**—Grown best on *hay infusion* of nearly neutral reaction. Medium diffusely clouded in six to ten hours, in twenty-four hours a pellicle, sometimes tough and whitish, sometimes merely a scum which is faintly iridescent. After two to three days a slimy deposit at the bottom of the tube arising in the form of a coarsely twisted rope on whirling. Growth in *broth* and *Witte's peptone* like that in hay infusion, but less abundant.

Growth in *litmus milk*, which is unchanged and shows no pellicle.

Growth on *nutrient agar* was at first difficult to obtain. After one to two generations the growth became profuse. The typical twenty-four-hour colony is round, convex, slightly flattened on top, 1 to 2 mm. in diameter with entire margin. Colonies mucoid in consistency and tend to coalesce. By reflected light they appear bluish-white and glistening. By reflected light under low magnification they are yellowish and finely granular. Colonies five to six days old where there is sufficient room for growth reach 6 to 7 mm. in diameter and show a distinct central boss. Deep colonies lenticular. Growth in *gelatin* like that on agar, without liquefaction. In a *gelatin stab* growth good on and near the surface, faint and scattered along the line of the stab. Growth not so abundant on Loeffler's serum as on agar or gelatin. No digestion.

On *potato* colonies are small, white, and slimy. No diastatic action on *starch*. Nitrates not reduced. No gas. No indol. No fermentation of *glucose*, *lactose*, *sucrose*, *maltose*, *mannitol*, *adonitol*, *rhamnose*, and *xylose*.

Optimum temperature about 30° C.

Guinea-pigs produce an agglutinating serum for the organism after six to seven injections of 2 c.c. twenty-four-hour broth cultures. This serum agglutinates *Vibrio percolans*

up to a dilution of 1 to 750, and has no effect on *Vibrio comma*, *Vibrio Metchnikovi*, and *Vibrio Finkler-Prior*.

Resistance fairly high, the organism surviving at least eight days at 0° C. in broth. Glucose broth cultures plugged with cotton viable after two months. Agar cultures sealed with paraffin viable after seven months. Hay infusion cultures plugged with cotton frequently alive after six months.

Non-pathogenic for white mice, white rats, wild rats, guinea-pigs, and rabbits by subcutaneous or intraperitoneal injection or by ingestion.

Passes through Berkefeld V, but not through Berkefeld N filters. Passage through Berkefeld V candles prevented by suppressing motility with acid, ether, chloroform, or with cold.

1. Mudd: J. Bact., 1923, viii, 459.
2. Mudd and Warren: J. Bact., 1923, viii, 447.

### VIBRIO AUREUS Weibel

Originally reported by Weibel[1] from canal mud as *Vibrio aureus*. Named *Spirosoma aureum* (Weibel) by Migula.[2]

**Morphology.**—Great variation in morplology except as to thickness which remains constant at about one and a half times that of the cholera vibrio. Commas and S forms somewhat plump with blunt ends are not so common as longer and shorter forms. The short forms are oval, but never true spheres (cocci). The regular comma forms may assume variable lengths, either as regular spirals with long-drawn out curves or as threads in which a spiral arrangement is seldom made out. The tendency to form curves seems to be less than with other vibrios. Degenerative forms common, especially in fluid media. They consist of small poorly developed commas, with badly stained ends, and chains of such commas loosely bound together. Long-drawn out S forms also appear, longer and shorter threads regularly notched and broken, of very irregular thickness. Peculiar rhombic rods also occur and among these irregular forms, sometimes the most beautiful regular spirals of extreme thinness.

Non-motile.

Gram-negative.

Grows at 22° C. and at 37° C.

**Cultural Characters.**—*Gelatin Plates.*—Deep colonies measure about 0.3 mm. in a few days, after ten days almost 1.0 mm. in diameter. The superficial colonies spread rapidly, are flat-convex, in three days 1 mm. and in ten days 3 to 4 mm. in diameter. Color, pure golden yellow. Under low power the deep colonies are asymetrical, broad, whetstone in shape, with fairly coarse granules. The center is at first golden-yellow, the edge lighter. Later a spindle-shaped thickening forms in the middle along the long axis, which is first brown, then finally black with the two edges remaining golden-yellow. Under low power the superficial colonies are circular with sharp edges, clearly granular, golden-yellow in the middle, paler at the edges.

*Gelatin Stab.*—Thick finely granular streak along the line of inoculation with coarser granules along the edge in old cultures. On the surface the growth spreads from the point of puncture till it attains a diameter of several millimeters and appears as a round top. Color, ochre-yellow in line and superficial growth.

*Agar Stab.*—Growth only on the surface at first as a dirty-white layer which gradually spreads over the whole surface to the edge of the test-tube. Inside this round raised yellow islands develop, and after increasing in size become confluent and form a homogeneous pulpy layer about 2 mm. thick. Color, golden-yellow to orange.

*Potato.*—Golden-yellow to orange growth, thick, abundant, and pulpy.

No special development in broth or Nägeli's solution. Turbidity and precipitate. No sediment.

1. Weibel: Centralbl. O. Bakteriol. [etc.], 1888, iv, 225; 257; 281.
2. Migula: p. 958.

### VIBRIO FLAVUS Weibel

Described by Weibel[1] from canal mud as *Vibrio flavus*. Named *Spirosoma flavum* (Weibel) by Migula.[2] It may be a variety of *Vibrio aureus* from which it differs only by the color of its growth. Non-motile. Gram-negative. Growth at 22° and 37° C.

*Gelatin colonies* like those of *Vibrio aureus*. Macroscopically the color is dirty gray-yellow on a dark background, straw-yellow on a white background. Under low power the deep colonies are light yellow, later golden-yellow, and uniformly colored, not deeper in the

middle. They are finely granular with fine interlacing markings. The superficial colonies are microscopically pale yellow with pale gray washed-out flecks and usually white peripheral zones. In *gelatin stabs* the surface growth is like that of *Vibrio aureus*, with a round top ochre yellow in color.

The growth on *agar* and *potato* is abundant ochre-yellow. Turbidity and sediment in *broth*. No scum.

1. Weibel: Centralbl. f. Bakteriol. [etc.], 1888, iv, 225; 257; 281.
2. Migula: p. 959.

### VIBRIO SAPROPHILES (Weibel)

Described by Weibel[1] as *Heuvibrio* a and subsequently as *Vibrio saprophiles* a. Named *Microspira Weibelii* by Migula.[2] Obtained from an infusion of hay or grass in which putrefaction had developed and from canal mud. This vibrio is much larger than Vibrio B.

**Morphology.**—Curved rods about 3 $\mu$ in length. Thickness in the middle about $\frac{1}{5}$ the length, but often the ends are pointed so that half-moon-like forms appear. Often the middle of the cell remains unstained as a light area. Frequently two organisms joined in S forms. Long chains are not common. In eight-day broth and agar cultures regular delicate curling threads, true spirals, appear. They consist of comma forms or S forms which appear most clearly in broth.

**Cultural Characters.**—*Gelatin Plates.*—Growth slow, but somewhat more rapid than Vibrio B. At the end of three days colonies have a diameter of 0.2 to 0.3 mm., at the end of six days a diameter of 0.6 mm., seldom more. Under low power (eighty times magnified) by transmitted light they appear as circular yellowish-brown disks. From the third or fourth day on darker concentric rings appear about a dark central point. In old colonies the edges of the deep colonies lose their sharp contour and seem to be eaten into. The superficial colonies form flat white-yellow expansions, under low power irregular, not circular. They show a sharply limited center and outside of this a grayish-yellow, finely granular zone poling toward the edges. No liquefaction. Old colonies are always lightly transparent.

*Gelatin Stab.*—Veil-like or cobwebby streak along line of inoculation. On the surface a whitish layer, gradually spreading, but not covering the entire surface. The uncovered surface seems to be permeated by a transparent whitish breath vapor. No liquefaction. Dirty yellowish red in old cultures.

*Agar Stab.*—Little development along the line of inoculation; occasionally a slight growth 1 cm. beneath the surface. Abundant growth on the surface, covering it with a creamy, dirty whitish-yellow expansion, underneath which the agar is rendered turbid to a depth of 1 to 2 mm.

*Broth.*—Fairly abundant uniform turbidity with a yellowish crumbly sediment after several days. In this wavy curling threads appear by microscopic examination.

*Potato.*—Abundant viscid slimy mass, yellowish-red in color after two days. This becomes darker and darker till it is chocolate-brown in color. At this stage it looks much like a culture of the glanders bacillus, but it is distinguished by its pulpy consistency and its deeper brown. Strong odor of ammonia.

*Staining Reactions.*—Staining well with anilin dyes. Decolorized by Gram.

Actively motile. No spores.

1. Weibel: Centralbl. f. Bakteriol. [etc.], 1887, ii, 465. *Ibid:* 1888, iv, 225; 257; 281.
2. Migula: p. 1005.

### MÜHLENS' ANAËROBIC VIBRIO

Cultivated by Mühlens from the mouth in horse serum agar (1–3). Small comma forms, smaller than the smallest forms of *Spirillum sputigenum* or the cholera vibrio. Stained by dilute carbolfuchsin or Giemsa the organisms appear as very small delicate comma-shaped reddish bodies. With Zettnow's flagella stain flagella are visible like those on *Spirillum sputigenum*, while the vibrios appear punctiform or as long ovals. The flagella are terminal. Not different from *Spirillum sputigenum* culturally. Serum agar stabs show slower and less abundant growth and less clearly marked cloudiness of the colonies. No odor of stinking putrefaction.

These organisms may represent some of Miller's mouth organisms. Probably different from *Spirillum sputigenum.*

Mühlens: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1909, xlviii, Orig., 523.

### VIBRIO TENUIS Veillon and Repaci

Found by Veillon and Repaci in ulcerating lesions in tuberculosis of the lungs and obtained in cultures by anaërobic methods.

**Morphology.**—In the pus and in tissues the organism appears as a short and extremely fine rod. One end is more pointed than the other so that the organisms look like commas. It is so small as often to be overlooked in preparations containing other organisms. In cultures on artificial media somewhat elongated, often end to end, and appearing like an S. In reality it is a very short spirillum with an elongated spiral.

*Motility* very active, the organisms starting and stopping abruptly like a spring, rotating rapidly on thin axes. In certain preparations so many organisms are moving at the same time that one gets the classic impression of a swarm of midgets.

**Staining Reactions.**—Stains well by Ziehl's fuchsin and Giemsa's solution. Gram-negative. Cilia stained by Loeffler's method. They are single, long, very fine, and attached to one end or along the body on the convex side.

**Cultural Characters.**—Growth only at 37° C.

*Agar Plates.*—Deep colonies appear as very fine granular points in forty-eight hours, small central masses surrounded by a halo. Under the microscope this looks like a central nucleus surrounded by a very fine cloud which seems to become thinner just at the point where one can distinguish the peripheral limit. Such colonies appear to the naked eye as very fine transparent clouds with more opaque central points. These colonies look like colonies of *Spirillum crassum*, but are smaller, finer, and more transparent. The opacity of the colony varies with the richness of the medium.

No gas. Fetid odor.

*Broth.*—Faint cloud which precipitates.

Very slightly resistant, requiring transfer every ten days.

Little or no pathogenic action.

Found normally in the mouth, also about carious teeth, dental abscesses, and in bronchial infections.

Cultivated in deep agar stabs of the following medium:

| | |
|---|---|
| Water, | 1000 c.c. |
| Agar, | 1.0 gram. |
| NaCl, | 5 grams. |
| Peptone, | 10 grams. |
| Glucose, | 15 grams. |
| Potassium nitrate, | 1 gr. |

Veillon and Repaci: Ann. d. l'Inst. Pasteur, 1912, xxvi, 300.

### SOPER'S ANAËROBIC VIBRIO

Isolated from a pericardial effusion by Soper and described as a spirillum.

Organism made up of segments lying end to end. They are 0.3 to 0.5 $\mu$ in width by 1 $\mu$ in length, ends blunt, waves regular and rectangular, wave-length averaging 1 to 2 $\mu$. Motility active, progressive and vibratory. Cultivated at first only anaërobically on the special media devised by Noguchi for *Treponema pallida*. Protein material not coagulated. No gas. Odor faint musty, not unpleasant. Subsequently the organisms grow aërobically. On *ascites agar* plates the deep colonies are opaque and punctate. Superficial colonies about 1 mm. in diameter, of regular outline and of an opaque grayish-white color. In all fluid media, covered by paraffin oil, ring growth of a faint color developed between the medium and the overlying oil.

Non-pathogenic.

Soper: Arch. Int. Med., 1913, xii, 273.

### TUNNICLIFF'S ANAËROBIC VIBRIO

Isolated by Tunnicliff[1] from the sputum of a case of acute bronchitis. A strict anaërobe. Isolated in pure culture after three days, incubation at 35° C. from a shining clear colony about 1 mm. in diameter, near the fluid of con-

densation of a tube of blood agar. In subcultures after two to three days inoculation pin-point colonies appear on the surface of blood agar. The growth is more glistening on alkaline blood agar than on plain agar. Profuse growth on the surface and in the fluid of condensation of Loeffler's blood-serum. No change on the surface, fluid of condensation rendered turbid. Faint growth along line of inoculation in ascites tissue agar stab and on ascites agar. No growth in milk, ascites broth or plain agar. No odor from the cultures. Successful cultivation in only a few of the tubes inoculated.

**Morphology.**—The vibrios measure 2 to 4 $\mu$ in length by $\frac{1}{4}$ $\mu$ in width. They show generally 1 or 2 curves, but sometimes more. Straight forms are also seen and chains of two or more vibrios. Ends generally pointed and not parallel to long axis. Sometimes a ring attached to one end. Rather thick long filaments also observed. Fine, long, wavy terminal flagellum stained by Zettnow's method.

Gram-negative. Stains fairly deeply with methylene-blue, not so intensely with carbol-gentian-violet or carbolfuchsin. Colored blue with Giemsa. Irregular staining frequently observed.

Under dark field illumination the vibrio is flexible and commonly very motile, not progressively, but rotating about its own axis with a corkscrew motion.

Pathogenicity not tested.

**Remarks.**—Differs from *Spirillum sputigenum* isolated by Mühlens[2] in the number and arrangement of the flagella and the staining reactions. *Spirillum sputigenum* generally possessing 2 to 3 flagella, one usually attached to its convex side and staining red with Giemsa. It also differs from *Spirillum crassum* and *Vibrio tenuis* of Veillon and Repaci[3] culturally in not producing any odor.

1. Tunnicliff: J. Infect. Dis., 1914, xv, 350.
2. Mühlens: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1909, xlviii, 523.
3. Veillon and Repaci: Ann. de l'Inst. Pasteur, 1912, xxvi, 300.

### GENUS 2. SPIRILLUM Ehrenberg, emended Migula

Cells, rigid rods of various thickness, length, and pitch of the spiral, forming either long screws or portions of a turn. Usually motile by means of a tuft of polar flagella (5–20) which are mostly half circular, rarely wavy-bent. These flagella occur on one or both poles; their number varies greatly and is difficult to determine, since in stained preparations several are often united into a common strand. Endospore formation has been reported in some species. Habitat: Water or putrid infusions.

Type species, *Spirillum undula* (Müller) Ehrenberg.

" *Kutscheri* Migula.
" *rugula* (Müller) Winter.
" *tenue* (Ehrenberg).
" *volutans* (Ehrenberg) Cohn.
" *serpens* (Müller) Winter.
" *giganteum* (Kutscher) Migula.
" *attenuatum* Warming.
" *amyliferum* van Tieghem.
" *rubrum* von Esmarck.
" *endoparagogicum* Sorokin.
" *concentricum* Kitasato.
" *desulfuricans* Beijerinck.
" *sporiferum* Migula.
" *leucomelænum* Perty.
" *rufum* Perty.
" *from fresh water* Dobell.

Organisms described as spirilla from the bodies of man or animals:

*Spirillum sputigenum* Miller.
" *nigrum* Rist.
" *crassum* Veillon and Repaci.
" *monospora* Dobell.
" from *Stylopyga orientalis* Dobell.
" *ostreæ* Noguchi.

Addenda

*Pseudospira serpens* Dobell.
*Paraspirillum Vejdovskii* Dobell.

### SPIRILLUM UNDULA (Müller) Ehrenberg

Spiral organisms from stagnant water and called *Vibrio undula* were originally described by Müller[1] and subsequently named *Spirillum undula* by Ehrenberg.[2] Much later, spiral organisms regarded as the same species were observed by Cohn, cultivated by Koch, and photographed by a number of observers. There is considerable doubt whether all these individuals were working with the same species. In 1895 Kutscher[3] described a spiral form from decomposing fluid as *Spirillum undula minus*. This is supposed to represent the original *Spirillum undula* of Müller and Ehrenberg. Description from Migula. Optimum temperature 25° to 30° C.

**Morphology.**—Fairly large spiral organism, usually with a half or whole spiral about 4 to 5 $\mu$ in height and breadth, rarely with spirals of 1½ to 2 or 3 turns. Motile from bunches of flagella up to 15 in number and attached at the poles.

**Cultural Characters.**—*Gelatin Plates.*—Colonies grow slowly, are round, with sharp edges, granular, light greenish-yellow, with the underlying gelatin slightly sunken.

*Agar Plates.*—Colonies whetstone in shape, coarsely granular, greenish-brown. The superficial colonies appear as greenish-brown, leathery expansions, with colorless jagged edges.

*Agar Stab.*—Whitish folded expansion at the point of puncture, cloudy turbidity in the upper two-thirds of the stab. The lower portion free from growth.

*Potato.*—No growth.

*Milk.*—No growth.

*Fluid Media.*—Turbidity, no superficial film.

1. Müller: Animalcula infusoria [etc.] 1786.
2. Ehrenberg: Infúsionstierchen. 1838.
3. Kutscher: Ztschr. f. Hyg., 1896, xx, 57.

### SPIRILLUM KUTSCHERI Migula

Obtained originally by Kutscher[1] as *Spirillum undula majus* from decomposing fluids. Supposed by Kutscher to represent the spiral organisms cultivated by Cohn[2] and called *Spirillum undula*. Called *Spirillum Kutscheri* by Migula.[3]

**Morphology.**—Curved organisms about a third larger than *Spirillum undula*, sometimes almost straight and sometimes forming long and narrow spirals.

Optimum temperature 22° to 27° C.

**Cultural Characters.**—*Infusion Agar Plates.**—Deep colonies round or whetstone in shape, dark brown with a light greenish shimmer, coarsely granular. When they break through to the surface they form a delicate round expansion over the agar. Under low power (60 diameters) this consists of delicately intertwined spiral forms. Later the colonies are thicker in the middle, yellowish-brown and opaque.

*Infusion Agar Slant.**—Delicate transparent colorless expansion in twenty-four to forty-eight hours.

*Infusion Agar Stab.**—Delicate thread-like growth along the line of the stab, with a slight expansion on the surface.

* Composition: Agar 1½ grams.
Sterile meat juice 100 c.c.
Made alkaline with soda solution.
Filtered and sterilized.

*Infusion Gelatin Plates* (neutral 10 per cent.).—Small round or whetstone colonies in three to four days, dark brown in color in the depths. Superficial colonies delicate transparent folded expansions. Under low power coarsely granular.

*Broth.*—Turbidity in twenty-four hours. No scum.

No growth on ordinary agar, gelatin, glycerin agar, blood-serum, etc.

1. Kutscher: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1895, xviii, 614.
2. Cohn: Beiträge z. Biol. der Pflanz. 1870, i, 181.
3. Migula: p. 1024.

### SPIRILLUM RUGULA (Müller) Winter

First observed by Leeuwenhoek in dental mucus, and by Müller[1] in diarrheal discharges, in stagnant water, and putrefying fluids. Named *Vibrio rugula* by Müller. Described subsequently by Cohn.[2] Description from Bonhoff.[3] Named by Winter.[4]

**Morphology.**—Very large organisms twice as thick as *Bacillus subtilis* and 8 to 16 $\mu$ long (Cohn). Slightly but definitely curved, usually like the bow of a violin, that is, with a flat curve in the middle and straight ends.

Cytoplasm smooth and containing granular masses.

*Motility* active. Rotation, boring and bending to both sides. When two organisms are attached the single individuals may move independently. The movements were likened by Müller to those of a swarm of bees.

*Stained* by anilin dyes, best by weak fuchsin in the cold with not too long an exposure. When stained by concentrated dyes unstained areas are left in the cells. Flagella may be stained by Loeffler's method. They are terminal, either single or skeins of several (8 to 15).

*Spores* were found in this species by Prazmowski, but it is not certain that his cultures were pure. Bonhoff observed spores once in eight-day cultures in peptone, but concluded that they were due to contaminating organisms. Pure cultures were destroyed by 60° C. in two and a half minutes and by drying on coverslips in four hours. Not very sensitive to organic acid.

**Cultural Characters.**—*Cultivated* by Bonhoff. Growth more rapid at 37° C. than at 22° C.

*Gelatin Plates.*—Deep colonies when young are elongated, oval, or roundish, with processes projecting from the edge, yellowish in color. Superficial colonies have some resemblance to anthrax colonies with the same curling threads on the edge. They are whitish with a slight cream color to the naked eye, slightly darker under the microscope. They are never round, but always irregular and jagged. On thinly seeded plates the gelatin colonies may slightly resemble colonies of *Bacillus coli*. No liquefaction.

*Gelatin Stab.*—Fine white expansion on the surface spreading from the point of puncture to the wall of the tube. Growth in the upper two-thirds of the stab, only isolated colonies below. No liquefaction.

*Agar Plates.*—Colonies appear in twenty-four hours at 37° C. They are grayish and leathery, difficult to distinguish from anthrax colonies. Under low power these colonies are seen to consist of organisms lying thickly against each other.

*Agar Slant.*—Abundant development at 37° C. in twenty-four hours.

*Glucose Agar Slant.*—Thick white surface expansion.

*Glucose Agar Stab.*—Good growth in the depths 1.5 cm. thick.

*Potato.*—No growth.

*Milk.*—No growth.

*Blood-serum.*—Abundant development of individual round whitish colonies of considerable thickness. No liquefaction.

*Broth.*—Slight turbidity.

*Peptone Water.*—Delicate sediment, no turbidity.

*Kutscher's Alkaline Broth Without Peptone or Salt.*—Turbidity in forty-eight hours with a thick scum on the surface.

Indol not produced.

Not pathogenic.

1. Müller: Animalcula infusoria [etc.], 1786.
2. Cohn: Berträge zur Biol. d. Pflanzen, 1872, i, 178.
3. Bonhoff: Arch. f. Hyg., 1896, xxvi, 162.
4. Winter: Die Pilze. 1884.

### SPIRILLUM TENUE Ehrenberg

First seen by Ehrenberg[1] in stagnant water. Subsequently investigated by Cohn.[2] Organisms identified as *Spirillum tenue*, found by Kutscher[3] and by Bonhoff.[4] Beijerinck states that there are three varieties of this species which can be differentiated by morphology. Present description from Bonhoff who had an organism much like Cohn's.

**Morphology.**—Organisms of hair thinness arranged as spirals with a height and diameter of 2 to 3 $\mu$. They may have a turn and a half, which gives a hook or S shape or 2 to 5 turns, 4 to 15 $\mu$ long.

*Motility.*—Very rapid, due to two terminal cilia on each end which may be demonstrated by Loeffler's flagella stain.

**Cultural Characters.**—*Gelatin Plates.*—Colonies in forty-eight hours small, irregularly round, dark brown with several large particles inside. The deep and superficial colonies are much the same except that the latter are larger. Surface colonies pure white to the naked eye, under low power yellow expansions spreading in all directions, without sharply defined edges, but not regular.

*Gelatin Stab.*—Rapid growth, whitish expansion on the surface, good growth along line of inoculation. No liquefaction.

*Agar Plates.*—Colonies appear in twenty-four hours at 37° C. Deep colonies like gelatin colonies of the cholera vibrio, made up of clear, very fine glass-like particles, irregularly round, but somewhat darker in color. Superficial colonies circular and thin with sharp edges, somewhat indefinite, yellow $\frac{1}{2}$ cm. in diameter, with large granules in the center.

*Agar Slant.*—Coherent gray-white mass in twenty hours. No scum on water of condensation.

*Glucose Agar Stab.*—Good surface growth at point of puncture. Growth in stab as a very fine line to the bottom of the tube.

*Blood-serum.*—Silver-gray expansion. No liquefaction.

*Potato.*—No growth.

Good growth in meat-juice broth, peptone water, and potato extract, with a thick film on the surface and a mass of bacteria at the bottom $\frac{1}{2}$ cm. thick. Good growth in 1 per cent. peptone solution containing $\frac{1}{2}$ per cent. NaCl.

*Milk.*—No very definite change, possibly more viscid.

*Nitroso-indol* reaction negative. Some *indol* in peptone solution.

Destroyed by drying on coverslips twenty-one hours, also at 60° C. in four minutes. Not affected by organic acids.

Non-pathogenic.

Bonhoff reports that this organism differs from Kutscher's isolation and is like one of Beijerinck's. Thus it does not liquefy gelatin and grows well in peptone solution. Kutscher's organism liquefies gelatin and does not grow in peptone. It has a terminal tuft of fine slightly wavy flagella.

1. Ehrenberg: Infusionstierchen [etc.] 1838.
2. Cohn: Beiträge zur Biologie der Pflanzen. 1872, i, 181.
3. Kutscher: Ztschr. f. Hyg., 1895, xx, 46.
4. Bonhoff: Arch. f. Hyg., 1896, xxvi, 162.

### SPIRILLUM VOLUTANS (Ehrenberg) Cohn

Demonstrated originally by Ehrenberg[1] and by Cohn.[2] Not cultivated. Because of its size employed for morphological investigations by many authors, especially Migula,[3] Bütschli,[4] Zettnow,[5] and Hoelling.[6] Common in decomposing fluids, stagnant water, etc.

Migula states that this organism forms long very regular spirals, usually about 30 $\mu$, but occasionally 70 $\mu$ long. The spirals are stiff and upright, the curves measuring 6.6 to 7 $\mu$ in diameter and 13 to 14 $\mu$ in height. Generally one individual has $2\frac{1}{2}$ spiral turns. The cells are $1\frac{1}{2}$ to 2 $\mu$ thick. Cell contents very granular (possibly containing sulphur). On the ends are tufts of 10 to 15 fairly long, slightly bent flagella. Movements intermittent, with clear spiral turning.

Bütschli, by the study of preparations fixed in alcohol and stained by Heidenhain's hematoxylin, has made out deeply staining areas inside the organism, which he regards as central bodies and light unstained ends. In the interior of the central body are many granules which are intense red violet in color. About the central body lies a peculiar narrow thread-like mass which may have spiral turns and an alveolar structure. This alveolar structure is

brought out better in preparations stained in gentian-violet. It is like the spiral sheath demonstrated by Zettnow in *Spirillum serpens* and *Spirillum undula* by Loeffler's flagella stain.

More recently Hoelling has shown that *Spirillum volutans* has a stiff rigid membrane like that of all plant cells. This may be demonstrated by the plasmolysis which occurs in 10 per cent. NaCl solution. In the interior lie metachromatic granules (volutin) which dissolve in 16 per cent. potassium hydrate. The plasma of the cells is alveolar. The motility depends upon two bundles of flagella, one on each end. These are long, penetrate the outer membrane through a hole and come in immediate contact with the cytoplasm. They are really single flagella, which may be split up into many delicate threads lying in a layer of plasma. There are no lateral flagella. Division transverse. Cell bodies rigid, not flexible.

1. Ehrenberg: Infusionstierchen [etc.]., 1838.
2. Cohn: Beiträge z. Biol. d. Pflanz., 1872, i, 181.
3. Migula: p. 1025.
4. Bütschli: Arch. f. Protistenk., 1902, i, 41.
———: Ueber den Bau der Bakterien und verwandter Organismen. Leipzig, 1890.
———: Weitere Unterssuchungen über den Bau der Cyanophyceen und Bakterien. Leipzig, 1896.
5. Zettnow: Centralbl. f. Bakteriol. [etc.], 1891, x, 690.
———: Ztschr. f. Hyg., 1897, xxiv, 72.
6. Hoelling: Arch. f. Protistenk., 1911, xxiii, 101.

### SPIRILLUM SERPENS (Müller) Winter

Described originally by Müller[1] in stagnant water and putrefying fluids as *Vibrio serpens*, and by Cohn.[2] Cultivated by Kutscher[3] from manure and water. Named by Winter.[4]

**Morphology.**—Fine spirals with 3 to 4 turns, 12 to 30 $\mu$ long, curves open, height of spiral 5 to 6 $\mu$ (Migula). According to Kutscher, this species tends to form swarming masses which may be observed regularly in hanging drops. Here they form one or more clumps which swim through the fluid. The individual cells occasionally show horn-like processes arising from the broad ends. No satisfactory evidence of true branching.

*Motility* active, due to bunches of 6 to 14 terminal flagella. Cultivated only on certain media. Optimum temperature 37° C.

**Cultural Characters.**—*Gelatin Plates.*—Greenish-yellow to brown coarsely granular round deep colonies with sharp edges. Superficial colonies develop as delicate expansions with light yellowish centers and colorless peripheries. The edges are very jagged. This expansion is made up of many curling strands of bacteria. Gelatin slowly liquefied.

*Agar Plates.*—Deep colonies usually whetstone in shape, yellow to brown, frequently provided with lateral protuberances. Superficial colonies spreading, yellowish in the middle, colorless in the periphery. Coarsely granular. The edges are sharp and broken by minute irregularities which under low power are seen to be the vibrios themselves.

*Agar Slant.*—Growth poor. Isolated colonies only with rich inoculation.

*Agar Stab.*—Growth only in upper part of stab.

*Gelatin Stab.*—Delicate white coherent thread of growth in the upper two-thirds of the line of inoculation. No development in the lower one-third. On the surface a white expansion with slightly notched edges. This slowly liquefies the gelatin and sinks into it, forming an air bubble.

*Potato.*—Thick, whitish, moist expansion.

*Milk.*—No growth.

*Agar Meat Broth.*—Turbidity and occasionally a delicate scum.

Non-pathogenic.

Destroyed at 65° C. in five minutes when dried on coverslips or on silk threads.

1. Müller: Animalcula infusoria [etc.] 1786.
2. Cohn: Beiträge z. Biol. der Pflanz. 1872, i, 179.
3. Kutscher: Ztschr. f. Hyg., 1895, xx, 46.
4. Winter: Die Pilze. 1884.

### SPIRILLUM GIGANTEUM (Kutscher) Migula

Obtained by Kutscher[1] from manure and identified as Ehrenberg's *Spirillum volutans*. According to Migula[2] this organism of Kutscher's is quite different from *Spirillum volutans* and it is, therefore, given a new name, *Spirillum giganteum.*

**Morphology.**—Great variation in size and shape, from straight rods to true spirals. In the interior of the cells are many discrete particles.

*Motility* active from a tuft of long, slightly wavy, terminal flagella up to 8 in number.

**Cultural Characters.**—*Cultivated* only on certain media. Optimum temperature 25° to 30° C.

*Gelatin Plates.*—Deep colonies round, greenish-brown and granular with sharp edges. Superficial colonies form a whitish expansion which under the microscope appears to consist of a greenish-brown coarsely granular center and a coarsely granular colorless very ragged edge. About the colonies the gelatin is a trifle sunken.

*Gelatin Stab.*—The upper two-thirds of the line of inoculation is filled with delicate whitish masses of bacterial growth, the lower third sterile. On the surface a porcelain-white very ragged expansion forms and gradually sinks into the medium.

*Agar Plates.*—Deep colonies granular, whetstone shaped. Superficial colonies resemble those of the diphtheria bacillus.

*Agar Meat Broth.*—Uniform turbidity. No scum.

*Potato.*—Grayish dry expansion.

*Milk.*—No growth.

Non-pathogenic.

Destroyed in five minutes at 65° C. when dried on coverslips or silk threads.

Swellengrebel,[3] who studied a large organism identified as *Spirillum giganteum* (*Spirillum volutans* Kutscher), reports them as 1.5 $\mu$ wide, 10 to 25 $\mu$ long, and containing many metachromatic granules (volutin of Meyer). These granules were visible in the living condition, together with fat particles and transverse bands forming a spiral or zig-zag filament. When stained with diluted methylene-blue a distinct alveolar arrangement could be made out. The alveoli had a diameter of 0.3 to 0.9 $\mu$ with very fine partitions between them. When stained with Sudan III certain of these alveoli, but not the majority took the fat stain. The spiral or zig-zag filament is regarded by Swellengrebel as a kind of nuclear thread containing granules of chromatin. The individual cells are easily plasmolyzed. Division transverse, the protoplasm thinning out in certain areas and taking the stain more deeply. Part of this deeply staining protoplasm becomes the terminal portion of the new cells. The flagella are terminal and arise from a kind of "calotte" which seems to be a portion of periplast lying on the ends of the organism. Involution forms common.

1. Kutscher: Ztschr. f. Hyg., 1895, xx, 46.
2. Migula: p. 1025.
3. Swellengrebel: Ann. de l'Inst. Pasteur, 1907, xxi, 448.
———: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1909, xliv, 529.

### SPIRILLUM ATTENUATUM Warming

Classified by Warming[1] as a spirillum and by Migula[2] as a spirosoma. From Migula's description this organism would now be regarded either as a spirillum or as a spirochete. It is a long spirally-turned organism with low, wide, heavy spiral turns in the middle and steeper turns at the ends.

The larger turns towards the middle are 11 $\mu$ high and 6 $\mu$ wide, the terminal spiral turns 10 $\mu$ high and 2 $\mu$ wide. The body is thicker in the center, about 2 $\mu$, and more slender at the ends, about 1.2 $\mu$. Body gray, finely granular. Non-motile.

1. Warming: Om nogle ved Danmarks Kyster levende Bakterier. Kjobenhavn, 1876.
2. Migula: p. 959.

### SPIRILLUM AMYLIFERUM Van Tieghem

Described originally by Van Tieghem[1] from the jelly-like masses of a fungus (Froschlaichpilz). Present description taken from Migula.[2]

Rigid spirals with 2 to 4 spiral turns of a diameter of 3 to 4 $\mu$ and height of 6 to 9 $\mu$. Thickness of cells 1.2 to 1.5 $\mu$. Spirals of 4 turns are divided in the middle. Before division the two halves bend toward each other until they are almost parallel much like a double mercury tube. The eventual division is produced by a mucoid transformation of the middle lamella of the intermediate wall. A fine jelly-like process remains attached to each new end. During development and division the cells may be stained yellow with iodin. Later when the longitudinal growth ceases the cell swells, refracts the light differently and stains blue with iodin, often up to white points at the poles. These changes precede spore-formation. The spores develop at these white points. They are darkly contoured ovoid bodies, 2.5 to

3.0 $\mu$ long and 1.5 $\mu$ wide. After the spores are formed the cells no longer stain blue with iodin. Such a cell is still capable of movement. Each cell forms one spore, and when a spiral of two turns has two spores a partition wall is visible in the middle. The spores vegetate by increasing in size and emitting a kind of germinal sheath, which is first bent and then spiral. The organism can grow without oxygen.

**Remarks.**—This remarkable organism is called a spirillum by Van Tieghem and by Migula. The mode of division is like that described by Gross as "incurvation" and the organism should probably be grouped with the spirochætes, possibly with the saprospiras.

1. Van Tieghem: Bull. Soc. botan. de France, 1879, xxvi, 65.
2. Migula: p. 1027.

### SPIRILLUM RUBRUM von Esmarck

Found by von Esmarck in water in which a mouse was allowed to putrefy. Later found by Adametz in water.

**Morphology.**—On solid media, short spirals with 1, 2 or 3 elements, but in broth long twisted chains of 40 to 50 elements. About twice as thick as the cholera vibrio. Some forms exhibit peculiar slimy spots in the bodies of the organism, especially evident in old cultures on solid media.

*Motility* active in the short forms, not noticeable in the long chains. Terminal bunches of flagella (Bergey).

**Cultural Characters.**—*Gelatin Slant.*—Surface colonies grow slowly, requiring six to eight days; young colonies show gray or pale-red centers under low power, granular with entire edges. The colonies later become wine red, especially those in the depths. No liquefaction.

*Gelatin Stab.*—Abundant growth along line of inoculation in the depth with wine-red color; near and on the surface the growth is white.

*Agar.*—Moist, slimy, gray-white expansion becoming wine red where it thickens.

Turbidity and red sediment in the water of condensation.

*Carbohydrates.*—No acid or gas.

*Blood-serum.*—Moist, slimy surface growth gradually becoming wine-red.

*Potato.*—Slow growth with the production of small wine-red colonies the size of hemp-seed.

*Broth.*—Turbidity and red sediment.

Destroyed in five minutes at 52° C.

Not pathogenic to mice, guinea-pigs, and rabbits.

von Esmarck: Centralbl. f. Bakteriol, 1887, i, 225.

### SPIRILLUM ENDOPARAGOGICUM Sorokin

Originally seen by Sorokin in decomposing rain water found on poplar tree trunks (*Populus nigra*). Not cultivated.

Spiral organisms with transparent cytoplasm free from granules. The young forms have generally two curves, the adult not more than three. They occasionally form moderately sized zoöglœa masses. Motility very active from terminal flagella, sometimes on one end and sometimes on both ends. The flagella may be demonstrated by weak iodin solution. Multiplication by simple fission. Old organisms are almost motionless and contain refractile spores. These spores begin to vegetate while still in the mother cell. Straight rods develop from the spores, and in fifteen to twenty minutes they begin to curve and separate from the cells. When still attached they give the cell an appearance of branching.

Sorokin: Centralbl. f. Bakteriol. [etc.], 1887, i, 465.

### SPIRILLUM CONCENTRICUM Kitasato

Found by Kitasato in putrid blood and by Lustig in water.

**Morphology.**—Small twisted spirals with pointed ends. Long threads with 5 to 20 turns or screws in broth cultures. The diameter of the screw measures 2 to 2.5 $\mu$, its length is 3.5 to 4 $\mu$. Slightly thicker than the cholera vibrio.

*Stains* well with aniline dyes.

*Motility* rapid with screw-like movements from bunches of polar flagella.

*Grows* best at 20° to 23° C., somewhat at 37° C.

**Cultural Characters.**—*Gelatin Plates.*—Pale-gray circular disks composed of concentric rings. The centers are white and opaque and are surrounded by transparent rings. These are in turn surrounded by opaque gray-white circles, again by transparent zones outside of

Fig. 37.—*Spirillum rubrum* from plain agar twenty-four hours old, stained with gentian-violet.

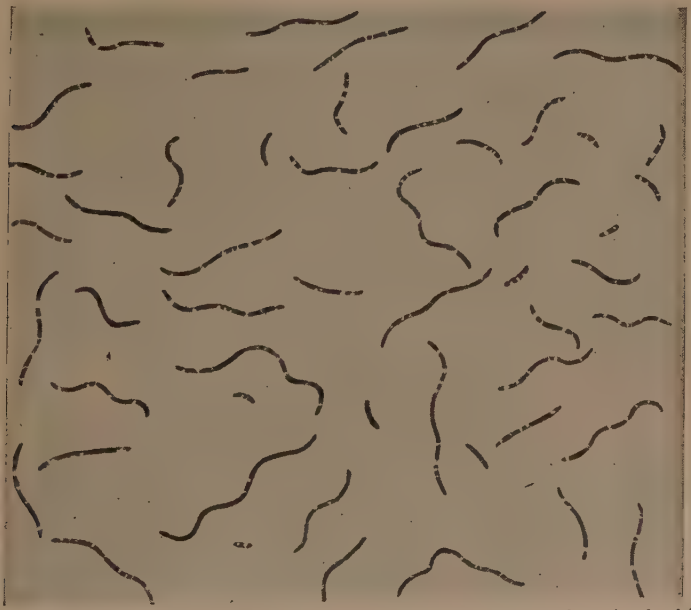


Fig. 38.—*Spirillum rubrum* from dextrose agar twenty-four hours old, stained with gentian-violet.

which are broad gray-whitish bands. Under low power the colonies show numerous small twisted prolongations. At the end of five days colonies measure 3 to 4 mm. in diameter.

No liquefaction.

*Gelatin Stab.*—Chiefly a surface growth, covering the top of the gelatin with a cloud-like expansion.

*Agar Slant.*—Diffuse surface expansion adhering firmly to the agar.

*Potato.*—No growth at 22° C. or at 37° C.

*Broth.*—Gradual production of a turbidity. In old cultures the broth clears and there is an abundant slimy deposit.

Non-pathogenic.

Kitasato: Centralbl. f. Bakteriol. [etc.], 1888, iii, 73.

### SPIRILLUM DESULFURICANS Beijerinck

Described as a sulfid-ferment, *Spirillum desulfuricans*, by Beijerinck.[1] Transferred to the Microspira by Migula.[2] Reduces sulphates to sulphids and forms a precipitate of black iron sulphid in the presence of iron salts.

*An obligate anaërobe* developing only in the presence of minimal quantities of organic matter, in consequence of which the reduction changes take place in solutions in which the nutritive material has been exhausted by bacteria. Isolated in a modified fermentation tube provided with a small tube leading into the closed arm. The anaërobic spirilla develop in the latter and a drop or two of the fluid containing them may be drawn out and cultivated. Also isolated in the presence of ordinary water spirilla which favor its multiplication, such as *Spirillum tenue*. Various synthetic media were used for isolation, as one of the following composition:

$\frac{1}{4}$ per cent. Potassium malate,
$\frac{1}{4}$ per cent. Peptone,
$\frac{1}{10}$ per cent. Moorsalts.

These are mixed with ditch water (Grabenwasser), then made alkaline with sodium carbonate and precipitated. This medium is now inoculated with *Spirillum tenue* and fresh ditch water (Grabenwasser).

In such tubes iron sulphid appears in twenty-four hours, and the organism producing this change, *Spirillum desulfuricans*, is found in the closed arm. Transfers now made to plates of special gelatin or agar media. The agar in aqueous solution is first washed in distilled water and mixed with a nutrient solution which contains sodium malate, asparagin, and potassium phosphate. During cooling a few drops of a clear neutral solution of moor-salts are added, and a trace of sodium carbonate. This medium is seeded with some of the mixed culture of the spirillum. The other bacteria present soon render it oxygen-free and then the sulfid-ferment develops. Sulphate reduction takes place and colonies are formed. The development of colonies can be favored by making agar plates and immersing them in solutions adapted to the sulphate reduction. In the presence of iron salts the colonies are easily recognized. They are either spheres of iron sulphid gradually merging peripherally with the medium or deep black points. Pure cultures may also be obtained in proper agar poured in layers between glass plates by inoculations with *Spirillum tenue* and *Spirillum desulfuricans*. The *Spirillum tenue* grows in one zone, the black colonies of *Spirillum desulfuricans* in another. The colonies contain small spherical black precipitates of iron sulphid. Colonies can also be obtained by a similar technic on gelatin which is not liquefied. The organism itself is a short, moderately wound spirillum about 4 $\mu$ long and 1 $\mu$ thick, with $\frac{1}{2}$ to 1 turn. Motility active, but only in the absence of oxygen. Motility due to a bunch of terminal flagella.

1. Beijerinck: Centralbl. f. Bakteriol. [etc.], 1895, i, 1; 49; 104.
2. Migula: p. 1016.

### SPIRILLUM SPORIFERUM Migula

Found once in decomposed beans and described by Migula.

Slightly spirally curved threads with 2 to 4 turns. Motility active from polar bundles of 10 to 15 cilia long and slightly bent. The spirals gradually become less active and finally motionless and show a numerous septation. The cells thus produced are twice as long as wide and a roundish oval spore forms in each. This is as wide as the cell, but only half as long. After spores are formed the mother cells disintegrate and form a slimy or viscid jelly in which the spores lie in rows as powerfully refractile granules. The spirals in which the spore-formation is beginning are like the organism described as *Spirillum leucomelænum* by Perty.

Not cultivated.

*Remarks.*—This and *Spirillum amyliferum* of Van Tieghem are the only spirilla in which spores have been observed.

Migula: p. 1028.

### Spirillum leucomelænum Perty

Found by Perty in stagnant water.

*Morphology.*—Short individuals joined end to end and forming spirilla with 2 to 3 turns. Contents deep black surrounded by clear aureole.

Perty: Zur Kenntniss kleinster Lebensformen. Berne, 1852.

### Spirillum rufum Perty

Found by Perty in well water in red mucous spots on the side of the well. These vary in color from rose-red to blood red.

*Morphology.*—Filaments 8 to 16 $\mu$ long, slightly reddish, with $1\frac{1}{2}$ to 4 spiral turns. It does not segment. Actively motile.

Perty: Zur Kenntniss kleinster Lebensformen. Berne, 1852.

### Dobell's fresh water spirillum

Described by Dobell from the water of the river Granta at Cambridge.

The larger forms are rigid actively motile spirilla with deep curves. They show 3 turns or less down to rather less than 1 complete turn. They are 1.25 $\mu$ in width and 30 to 60 $\mu$ in length. Both ends have a single long slender flagellum, which may be seen in living material or in preparations stained by Heidenhain. The ends are round and stain deeply. No crista or similar structure. Circular in optical transverse section. The living organisms possess a thick and highly refractile investing membrane and are often filled with metachromatic granules. The cytoplasm is distinctly chambered. This may be made out in individuals fixed in Flemming's fluid, Schaudinn's sublimate alcohol or Bouin's fluid, and stained by Heidenhain. From end to end the organism consists of a single row of protoplasmic chambers or alveoli, like those of *Cristispira veneris* and other spirochætes. When deeply stained the chambers appear to have thick walls. If properly differentiated the walls appear very delicate, and tiny granules can be seen at the points where transverse partitions meet the body wall. Surrounding each transverse cytoplasmic septum is a ring of darkly staining granules, which are regarded by Dobell as nuclear, a modified form of chromidial nucleus. In dried Giemsa preparations the chambers break up, the granules disappear in the cytoplasm, producing "chromatin" bars and zigzag filaments like those in cristispiras, pseudospiras, etc. These granules take a red color like chromatin. Other granules occur which are probably metachromatic granules or volutin. They stain red with neutral red (intravitally) and also red with methylene-blue, thionin, or Delafield's hematoxylin. They lie in the transverse protoplasmic septa just as in saprospiras or pseudospiras. They stain red with Giemsa, black with Heidenhain, readily decolorizing with iron-alum solution. They usually appear grayish or white. Division transverse. Cell bodies rigid without true flexion. No spores.

The smaller forms have a breadth of about 0.6 $\mu$ and a length of 8.20 $\mu$ and always occur as a much extended spiral. Body rigid. Characteristic spirillar motility. Division transverse. No metachromatic granules. Distinct chambered structure like that seen in the larger spirilla. The alveoli or chambers are arranged in a single regular row regularly distributed.

Dobell: Quart. J. Mic. Sc., 1911, lvi, 395.

### Spirillum sputigenum Miller

Described originally by Miller[1] from the mouth and cultivated by Mühlens.[2]

**Morphology.**—Comma-shaped organisms and half-moon forms, thicker and longer than the cholera vibrio. Often two organisms are fastened together as an S. Larger forms with pointed ends may resemble the smaller slightly curved forms of *Bacillus fusiformis*.

*Motility* fairly active, vibratory, rotating, whirling, and boring. Hoffman and Prowazek[3] claim that *Spirillum sputigenum* has peritrichous flagella. Mühlens reports 1 to 3 flagella, the majority of the organisms having apparently a single thick flagellum (a bunch of flagella) on the concave side. Many forms show a second flagellum on the other side and many individuals have 3 fine flagella. In the last it is impossible to determine whether the flagella arise from the concave side alone or from both sides.

Stained well with dilute carbolfuchsin and gentian-violet. Pale red with Giemsa.

**Cultural Characters.**—*Cultivated* by Mühlens from material between the teeth in his special medium, horse serum agar (1 to 3), in deep shake tubes and stabs. Fine hazy colonies develop in the lower portion like the colonies of *Spirillum dentium* (*Spirochæta dentium*). In stab cultures growth begins in one to three days as fine cloudy colonies with somewhat thicker yellowish centers. The growth increases to a thicker streak, opaque in the center and cloudy transparent at the edges. Growth exclusively anaërobic and in only the lower two-thirds of the medium. Later transfers were successful to deep tubes of neutral, weak and strongly alkaline agar and grape sugar agar without serum. In highly alkaline agar the growth was slower and less abundant, without the usual cloudiness. Growth good in Kutscher's placenta agar.[4] No growth in serum broth. Cultures free from putrefactive odor.

1. Miller: Die Mikroörganismen der Mundhöhle. Leipzig, 1892.
———: Deutsche med. Wchnschr., 1906, xxxii, 1, 348.
2. Mühlens: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1909, xlviii, 523.
———: Deutsche. med. Wchnschr., 1906, xxxii, 1, 797.
Mühlens and Hartmann: Ztschr. f. Hyg., 1906, lv, 81.
3. Hoffmann and Prowazek: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1906, xli, 741.
4. Kutscher: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1908, xlv, 286.

### Spirillum nigrum Rist

Found by Rist in suppurative material from the ear.

Small, thin, curved, actively motile organisms, motion wave-like, very rapid, Motility lost in the neighborhood of air bubbles. The majority of the organisms have black granules either in the middle or on the ends which slightly swell the body. Stains easily with anilin dyes, best with Ziehl's dilute fuchsin solution which reveals fine granules in the body.

Decolorized by Gram.

Strict anaërobe. Growth only at 37° C.

Colonies in sugar agar appear in twenty-four hours, later quite large, 2 to 3 cm. in diameter. They are lentil-shaped, coal-black, quite opaque, occasionally gray or brownish black. Masses of small cultures gray. Colonies in gelatin opaque black without liquefaction. Dark gray turbidity in broth with dark precipitate. Cultures very fetid, with an odor like $H_2S$. Scanty gas formation. Cultures live over a month.

Pathogenic for guinea-pigs, more toxic than infectious. Animals die in fourteen days without lesions.

Rist: Centralbl. f. Bakteriol. [etc.], 1901, xxx, 287.

### Spirillum crassum Veillon and Repaci

Obtained by anaërobic methods from ulcerative lesions of the lung in tuberculosis by Veillon and Repaci.

**Morphology.**—In the pus and in infected tissues a very tapering spindle, usually curved, often in the form of a crescent. When two organisms are attached end-to-end their curves are inverse so that S forms appear.

*Motility* active in fresh material, rotating and changing in position.

In cultures analogous forms are present, but they are apt to be elongated and filaments are found like spirals with close, extremely short curves. Organisms in cultures are extremely motile, moving from place to place with great rapidity, often advancing and retreating. Motionless forms often have almost a spring-like motion in starting and stopping. Motility due to very long and very fine cilia, attached to the ends and along the sides.

**Staining Reactions.**—Stains by gentian-violet, Ziehl's fuchsin and by Giemsa.

Gram-negative.

**Cultural Characters.**—*Agar Plates.*—Colonies appear in forty-eight hours at 37° C. as fine points barely visible to the naked eye. Under low power spherical masses, yellowish and very granular. Later this mass becomes deeper yellowish, the borders are smooth, undulating and shade off abruptly. To the naked eye such colonies appear like white masses very finely clouded. This cloudiness is more or less cottony in aspect according to the richness of the agar.

*Broth.*—Less abundant growth. Delicate turbidity and sediment at the bottom as the broth clears. No growth except at 37° C.

No gas. Cultures fetid.

Cultures have little vitality and must be transferred every four to five days.

Slightly *pathogenic* for the rabbit. Produces gangrenous abscesses beneath the skin in guinea-pigs which occasionally die.

Found also in a putrid pleurisy and in the mouth. This may be the spirillum associated with *Bacillus fusiformis* in Vincent's angina.
Note: This organism may be a vibrio and not a spirillum.

Veillon and Repaci: Ann. de l'Inst. Pasteur, 1912, xxvi, 300.

### Spirillum monospora Dobell

Described by Dobell from the large intestine of frogs and toads. Not infrequent.

**Morphology.**—Usually S-shaped, 6 to 8 $\mu$ in length by about 1.5 $\mu$ in breadth. Actively motile. In deeply stained iron-hematoxylin preparations a flagellum (or bunch of flagella) may be found at either end. No simple nucleus or nuclear spiral present, but a number of granules like those found in *Spirillum volutans* by *Bütschli*. These granules stain in a manner suggesting that they are chromatin.

Transverse division as in other spirilla. No definite division of nuclear granules made out.

**Spore-formation.**—The nuclear granules, which in the vegetative stages are scattered throughout the cell, begin to aggregate at one end, producing a darkly staining nucleus-like mass. A spore membrane appears around this and as it hardens it gradually loses its staining capacity so that finally an unstained highly refringent spore becomes visible at the end of the organism. Only a part of the granules enter into the formation of the spore. The remainder gradually degenerate and are possibly in part volutin and other non-nuclear substances. Ultimately the rest of the cell, without the spore, breaks down so that the single spore is left with more or less of the dead cell attached. Spore germination not observed.

Motility remains until the spore is fully formed, the organism swimming about bearing its terminal glistening spore.

Dobell: Quart. J. Micr. Sc., 1908, lii, 121.

### Dobell's Spirilla from Stylopyga orientalis

Two types of spirilla have been reported by Dobell from the cockroach (*Stylopyga orientalis*). Form A, the largest, has a length of 5 to 16 $\mu$ and 1 to 3 complete turns. The width is 1.7 $\mu$ and it may appear thicker in stained preparations. The spiral is closely wound with an exceedingly thin pellicle. The axis of the spiral shows a curious thickening in some individuals which may be compared with a newel in a spiral staircase. This is called an *axial fiber* by Dobell. The cytoplasm has an irregular alveolar structure with a few deeply staining granules at the nodes. These alveoli do not lie in a single row, as one alveolus may not extend completely across the spiral. Long organisms may in whole or in part contain a single row of alveoli throughout the entire length with the exception of the extreme upper end. Darkly staining granules occupy nodes of the alveolar walls, that is, they are placed at the point where transverse partitions join the body wall. The protoplasmic structure is thus like that of the cristispiras or other spirochetes. Form B was originally described by Dobell as the "large spirillum from stylopygæ." It is smaller than Form A. The short individuals possess protoplasm of the usual alveolar structure, while the long forms show a distinct chambered structure extending for a variable distance along the spiral. This is a modified form of the irregular alveolar cytoplasm which is usually present and is like the structure of the cristispiras. The short individuals do not possess a chambered structure, but often one or two large cystoplasmic alveoli can be seen toward the center of the body. A few individuals show an axial fiber like that in Form A. In addition to Form A and Form B other spirilla occur intermediate in size and showing well-marked axial fibers. All these spirilla are easily plasmolyzed.

Dobell: Quart. J. Micr. Sc., 1911, lvi, 395.

### SPIRILLUM OSTREÆ Noguchi

Described by Noguchi in oysters.
Measures 8 to 16 $\mu$ in length and 0.5 $\mu$ in its widest part.
Both ends taper off to fine filaments.
Number of spirals 4 to 8. Curves shallow and very regular.
Body elastic but not markedly flexible.
Organisms rotate rapidly, but seldom progress. Not cultivated.

Noguchi: J. Exper. M., 1921, xxxiv, 295.

## ADDENDA

### GENUS PSEUDOSPIRA Dobell

Established by Dobell for the colorless free-living forms not adequately described heretofore. Probably the same as those placed in the genus *Vibrio* Ehrenberg by Cohn in 1872, and emended by him, but this is not certain.

Type species *Pseudospira serpens* Dobell.

### PSEUDOSPIRA SERPENS Dobell

Obtained by Dobell from the ooze and decaying vegetable matter found in the river Granta at Cambridge. The organisms live only a few days in laboratory cultures.

**Morphology.**—Long, slender, very flexible filament which tapers very slightly toward both ends. Filament cylindrical, *i. e.*, circular in optical transverse section. Length 16 to 66 $\mu$, average about 35 $\mu$. Width about 0.8 $\mu$ in all specimens. Not a true spiral. No crista or similar structure.

**Motility.**—The organism moves very rapidly with a graceful snake-like motion. Body extremely flexible and thrown into a variable number of waves which pass quickly along it from before backward, during progression. No antero-posterior polarity. During movements, the waves which pass along the body often make the organism appear as a spiral at first sight. Body really never a true spiral, always a very supple and much curved rod. No appreciable rotation. When not moving the organism may be perfectly straight like a very long bacillus. It may also bend in any direction till it is looped or doubled on itself. Ends frequently oscillate rapidly. Slowly rippling waves may pass from one end to the other in motionless individuals. Movements like that of a snake when swimming. Progression also by cilia. These are very fine and form a complete coat covering the whole organism. Not seen in living forms. Demonstrated by Loeffler's flagellar stain or iron hematoxylin.

**Internal Structure.**—Organisms colorless and protoplasm hyaline in unstained material. No definite structure, due perhaps to a very refractive membrane investing the body. Few relatively large and highly refractive granules may be seen in the living forms. After fixation and staining a well-marked structure can be made out, like that of *Cristispira veneris*. (Fixation by fluids of Flemming, Hermann, Bouin, Schaudinn, picro-acetic, acetic-alcohol, sublimate-acetic. Staining by Heidenhain's iron-hematoxylin, Delafield's hematoxylin, Giemsa, and methylene-blue.) The organism is divided from end to end into a single series of very definite cytoplasmic chambers or alveoli. Transverse partitions separating the chambers are irregularly disposed along the organism so that the chambers are of unequal size. Septa or partitions also appear to be of variable thickness. Dark chambers like those in *Cristispira veneris* also seen. The chambered structure is very distinct in specimens stained with iron-hematoxylin or methylene-blue. The refractive granules seen occasionally in living organisms are metachromatic granules. They occupy a position in the middle of the transverse protoplasmic partitions. They stain red with methylene-blue, and are often invisible in iron-hematoxylin and Giemsa preparations. Internal structure apparently like that in *Cristispira veneris*. Dried Giemsa preparations shows "chromatin" figures like those in Cristispiras. The walls of the chambers or alveoli break down or run together to form darkly staining granules, rods, short bent or zig-zag filaments, etc. Such structures are never encountered in properly fixed wet film preparations.

**Plasmolysis** occurs in strong NaCl solutions (10 per cent.) like that in the saprospiras. The membrane separates from the protoplasm at several points and swells out into vesicles. This frequently occurs at the end of the organism as in saprospiras. The vesicle away from the ends may also frequently divide its protoplasm completely across so that a kind of large bubble may distend the middle region of filament. Body evidently surrounded by extensible membrane and not multicellular.

**Division** transverse into two elements. It takes place in the middle of the body where a transverse protoplasmic septum occurs. The body is constricted at this point until only a very small slender thread remains between the daughter individuals. Division like that in the saprospiras. No multiple segmentation. No spore-formation.

Several other species of pseudospira have been observed by Dobell, also from fresh water. They were smaller than *Pseudospira serpens*, actively motile from peritrichous flagella and divided transversely. In some cases a distinct chambered structure could be made out.

Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

### Genus Paraspirillum Dobell

Established by Dobell for a new species presenting certain unusual features, especially the flexibility of the organism and the presence of a nucleus. Type species *Paraspirillum Vejdovskii* Dobell.

### Paraspirillum Vejdovskii Dobell

Found by Dobell in the fresh water of the river Granta at Cambridge.

**Morphology.**—Living organisms occur in the form of spiral or S-shaped rods very like ordinary spirilla. Length 8 to 25 $\mu$, average 15 $\mu$. Breadth variable. The organisms show a well-marked thickening toward the middle of the body and taper off markedly toward the ends, like a much elongated and spirally twisted spindle. In the middle region the organisms measure from about 1.5 to 2 $\mu$ across, the thickness varying in different individuals. In every individual an oval or rounded structure occurs in the middle of the organism where the body is thickest. This is a *nucleus*. The cytoplasm about it is always hyaline or very finely granular, quite free from any granular inclusions. Outside this area of clear protoplasm, between it and the ends of the organism, a number of highly refractile metachromatic granules, of variable size and distribution, can always be seen. No alveolar or other structure made out in the living organisms.

**Movements.**—Locomotion effected in the screw-like manner characteristic of spirilla. The organism remains in the form of an apparently rigid spiral and then moves rapidly with screw-like movements in the direction of the long axis of the body. No anteroposterior polarity. The organism also moves by rotation about a fixed end, making almost a complete revolution, or oscillating and moving back to the point from which it started. While the body remains rigid during the screw-like movements, it can bend, assuming the form of a very strongly curved spiral or can straighten itself out until it becomes almost rod-like. No true rods are ever formed, however, the organisms never straightening completely. Locomotion is effected by means of flagella similar to those of ordinary spirilla. While not seen in living organism they can be demonstrated by fixing wet films in sublimate and acetic acid (saturated solution $HgCl_2$ + 5 per cent. acetic) and staining very deeply with Heidenhain's iron-hematoxylin, both iron-alum and hematoxylin solution being allowed to act for many hours. The organisms show one or two excessively delicate flagella at either end. This "paraspirillum" thus resembles many other spirilla in flagellation.

**Nucleus.**—The nucleus seen in unstained individuals can be demonstrated by the use of a dilute solution of neutral red applied intravitally. At first only the metachromatic granules stain red, the nucleus remaining colorless and the organism remaining motile. Later the organism dies, the cytoplasm becomes pink and the nucleus takes up the stain, the cytoplasm turning pink, the metachromatic granules and the nucleus deep red. This nucleus thus behaves towards the dye in exactly the same way as the nuclei of minute amebæ and flagellates in the same preparation. The organisms also show marked vacuolation of the cytoplasm and appear somewhat swollen. The nucleus can likewise be demonstrated by fixing in Bouin's picro-formol-acetic fluid or sublimate and acetic acid and then staining with Heidenhain's iron-hematoxylin, methylene-blue, or Giemsa. In the Bouin-Heidenhain preparations the nucleus is well seen in all the individuals, round, oval, square, or oblong. It often appears to occupy the whole width of the organism. It is usually deeply stained and appears quite homogeneous or shows a distinct karyosome. Such organisms suggest that the nucleus is probably a vesicular structure and not merely a homogeneous mass of chromatin substance. Other organisms suggest the presence of a nuclear membrane. In Bouin-methylene-blue preparations the nucleus in every individual is stained deep blue, the metachromatic granules red. In organisms fixed in Bouin's fluid and stained by Giemsa the nucleus is stained red. No karyosome is demonstrated in organisms stained by Giemsa or methylene-blue. In broad individuals the cytoplasm about the nucleus takes up the chromatic stain, staining red with Giemsa.

*Nuclear Division.—Paraspirillum Vejdovski* divides transversely into two daughter

individuals like ordinary spirilla. The division of the body is preceded by the division of the nucleus. The originally oval nucleus undergoes a constriction in a plane at right angles to the long axis of the body. The two parts then separate to a short distance, remaining attached by a short strand of nuclear material giving rise to a dumb-bell figure. The connecting thread then vanishes and the two daughter nuclei appear as deeply stained, completely separate, somewhat hemispherical masses. They then draw further apart and constitute the nuclei of the new daughter individuals.

The metachromatic granules stain red with neutral red (intravital staining), red with methylene-blue, red or pink with Giemsa, but not so darkly as the nucleus. In Bouin-Heidenhain preparations they are at first deeply stained, but give up the dye on differentiation more readily than the nucleus. In well differentiated preparations they are mostly colorless while the nucleus remains deeply stained. They behave exactly like metachromatic granules in bacteria, yeasts, cyanophyceæ, and bacilli. They are confined to the ends of the organism and vary greatly in size and number. The cytoplasm shows no distinct structure in living material and in Bouin-Heidenhain preparations. Stained by Giemsa or methylene-blue it shows a very clear alveolar structure.

Dobell: Arch. f. Protistenk., 1912, xxiv, 97.

## FAMILY IV. COCCACEÆ ZOPF, emended Migula

Cells in their free conditions, spherical; during division somewhat elliptical. Division in one, two, or three planes. If the cells remain in contact after division they are frequently flattened in the plane of division, and form chains, packets, or irregular masses. Motility rare. Endospores absent. Metabolism complex, usually involving the utilization of amino-acids or carbohydrates. Pigment often produced.

### TRIBE A. NEISSEREÆ, Nov. Trib.

Strict parasites, failing to grow or growing very poorly on artificial media. Cells normally in pairs. *Gram-negative.* Growth fairly abundant on serum media.

#### GENUS 1. NEISSERIA Trevisan

Generic characters those of tribe.

Type Species *Neisseria gonorrheæ* (Neisser) Com.
" *intracellularis* or *meningitidis* (Weichselbaum).
" *catarrhalis* (Pfeiffer).
" *crassa* (Kutscher).
" *sicca* (v. Lingelsheim).
" *flava* (v. Lingelsheim).
" *perflava* Bergey.
" *subflava* Bergey.
" *mucosa* (Elser and Huntoon).
*Diplococcus intracellularis equi* Johne
" *albus tardissimus* Eisenberg.

#### NEISSERIA GONORRHŒÆ (Neisser) Com.

Discovered by Neisser[1] in 1879 in gonorrheal secretions and described by him as a biscuit-shaped diplococcus with the flat sides approximated, usually within the cells. Previous to Neisser's work organisms had been found in blepharitis which we now believe to have been gonococci, and the condition had been shown to be infectious, material from a case of blepharitis producing the same lesions when introduced into normal eyes.

First cultivated by Bumm[2] on placenta blood serum, beef serum, and sheep serum and by Wertheim[3] on serum agar. Usually known as the "gonococcus" of Neisser.

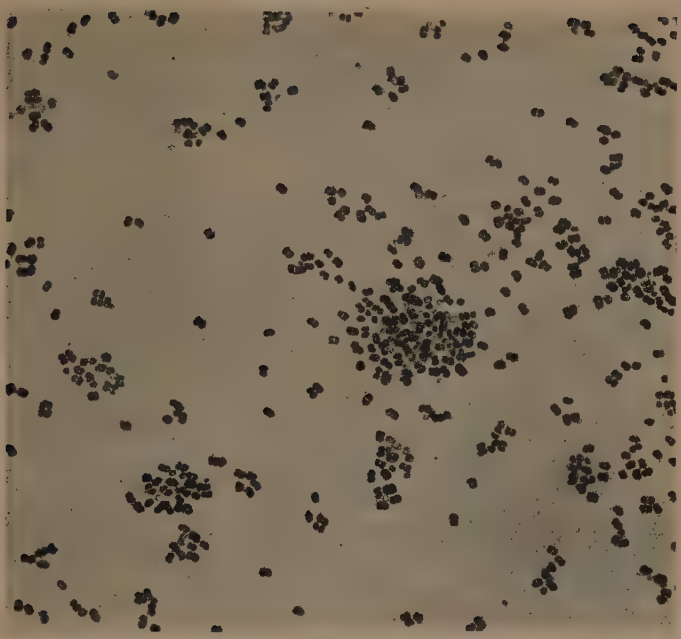


Fig. 39.—Gonococcus from twenty-hour culture on blood agar, stained with carbolfuchsin.

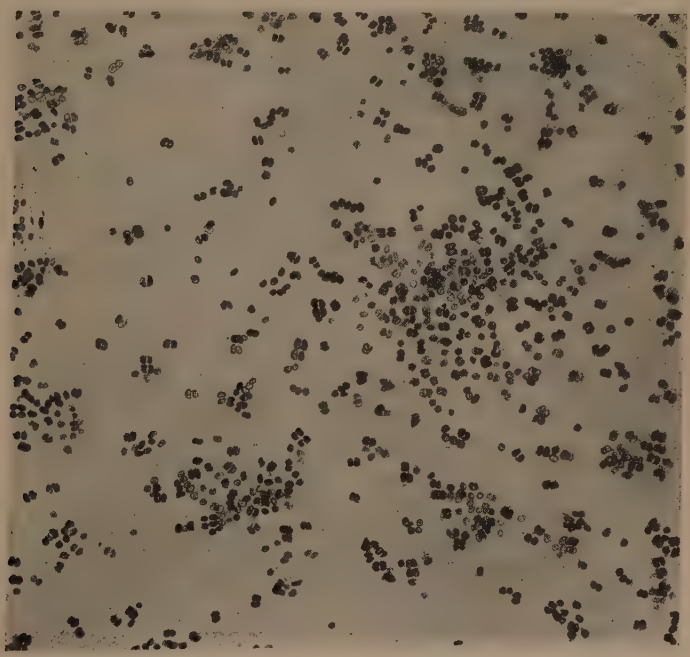


Fig. 40.—Gonococcus from forty-eight-hour culture on blood agar, stained with gentian-violet.

**Morphology.**—In the secretions from urethritis, vaginitis, gonorrheal conjunctivitis, etc., the gonococcus occurs as a diplococcus described as biscuit-shaped or coffee-bean-shaped, each element being disk-shaped. Spherical forms also occur. Division takes place by a central constriction, a single coccus dividing into two, these two into two more. Occasionally the second plane of division is at right angles to the first, and tetrads are formed. Individual cells are about 0.8 $\mu$ in breadth by 1.6 $\mu$ in length (Bumm), young forms being somewhat smaller. With alcohol preparations the organisms are much smaller. Deeply stained organisms often look larger than the lightly stained.

The gonococcus has a characteristic intracellular position inside the leukocytes. The organisms lie inside the protoplasm as may be demonstrated by treating fresh gonorrheal pus with acetic acid (Bumm). When stained with intravital stains like neutral red (1 c.c. cold saturated solution in 100 c.c. physiological salt solution), the intracellular organisms appear deep red, the extracellular not being stained. The number inside the cells varies from 2 or 3 to 100 (Neisser and Scholtz[4]) or to 200 to 300 (Leistikow[5]). The cells are often much swollen and may burst.

Gonococci reach their intracellular position as a result of the phagocytic action of the cells, not from their own activity, being non-motile. Inside the leukocytes they are still

Fig. 41.—Gonococcus in film from urethral pus, stained with methylene-blue.

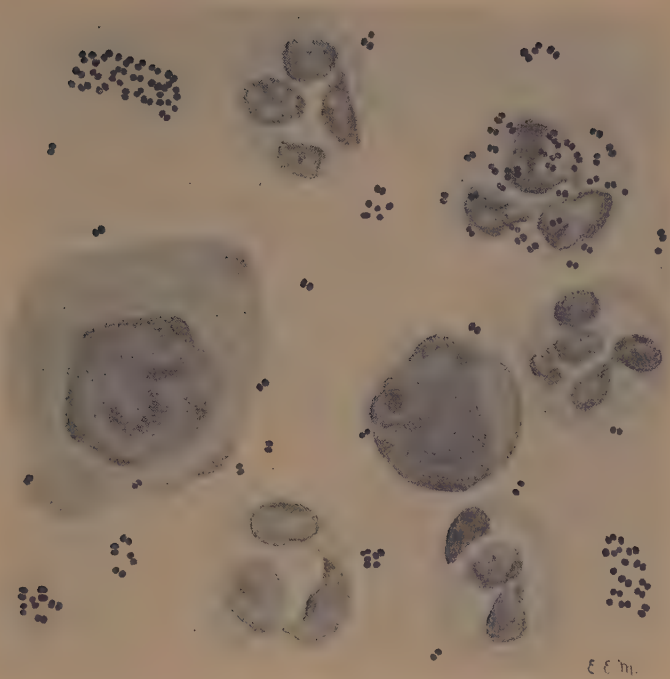
capable of growth (Bumm, Baumgarten), their morphological and biological properties not being injured. According to some authors the organisms may be inside the nuclei (Bockhart,[6] Haab,[7] etc.), but this is not accepted by Neisser, Bumm, or Jadassohn.[8]

Gonococci also lie free in the secretion and then appear usually in bunches. They may lie on the epithelial cells which may be plastered with them, especially in the early stages of urethritis. It is not entirely clear whether they are also engulfed by the epithelial cells acting as phagocytes or remain on the outside.

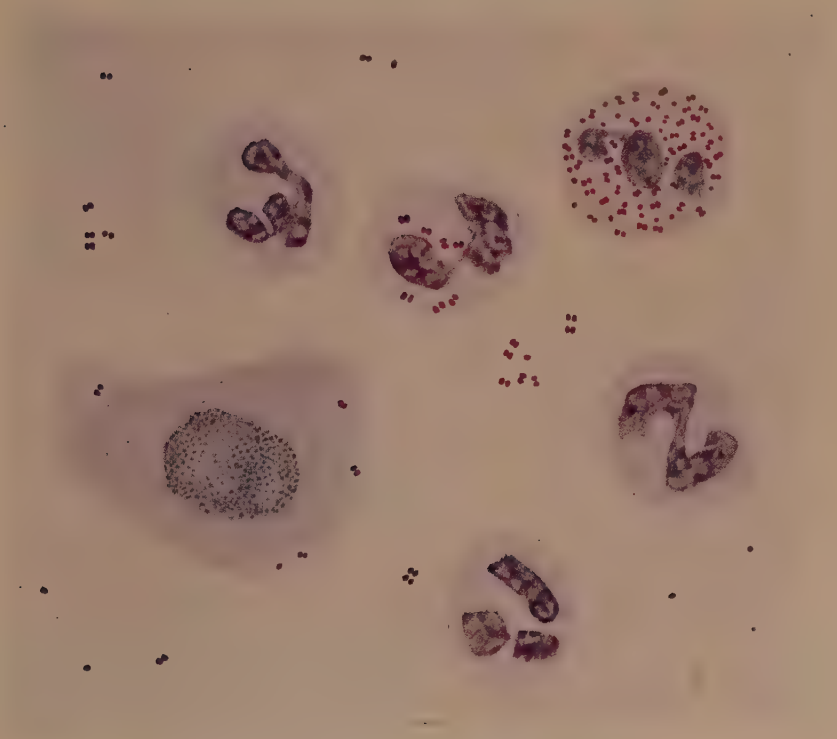
In cultures the organisms are smaller, especially when stained by alcoholic solutions of dyes. On blood agar cultures twenty hours old they measure about 0.4 by 0.5 $\mu$ or 0.5 by 0.6 $\mu$. In cultures they appear as single elements, diplococci, tetrads, and bunches, but diplococci are not so common as in the secretions. In old cultures degenerated forms are common, swollen, and staining irregularly.

Dissolved in sodium taurocholate.

**Staining Reactions.**—The organisms stain well by the usual anilin dyes. In pus they may be demonstrated best by Loeffler's methylene-blue, the protoplasm of the cell appearing pale blue, the cocci dark blue or blue-black. They stain well with dilute aqueous solutions of carbolfuchsin, safranin, Bismarck brown, etc. With gentian-violet they are apt to appear



Gonococcus from urethral pus, stained with methylene-blue.



Gonococcus from urethral pus, stained by Gram.

distorted. They also stain well in leukocytes by Giemsa, the cocci appearing blue, the protoplasm of the cell red. Gram-negative, both in pus and cultures, decolorizing easily in alcohol, often in two to three minutes. In the tissues the gonococci stain best with concentrated methyl violet in toluidin or anilin water applied a half hour and differentiated in alcohol (Bumm), Nicolle's dilute carbol methylene-blue differentiated in 1 per cent. tannin, Loeffler's methylene-blue, washed with distilled water and followed by dilute eosin, alcohol and oil of lavender.

**Cultivation.**—The gonococcus does not grow on ordinary media, requiring proteins from the human body like blood-serum, ascitic fluid, hydrocele fluid, or the sera of animals, beef, sheep, etc. It grows for one generation when pus is planted on ordinary agar, the proteins of the pus sufficing for the first growth. It grows sparsely on coagulated human serum, but may be cultivated on media containing the fluid from ovarian cysts, hydrosalpinx and hydrothorax. A great many different media have been devised for the cultivation of the organism, different bacteriologists preferring different combinations. Growth only between 30° and 39° C. Optimum temperature 36° to 37° C. Growth appears in twenty-four to forty-eight hours and does not increase after four to five days.

*Hydrocele Fluid Agar.*—This is in many respects the best medium. The hydrocele fluid may be added to melted agar in various proportions, 1 to 4 or 5, after which the agar is poured into plates or tubed. For isolation the pus may be smeared over the surface of plates. After twenty-four hours at 37° C. the colonies are small, round, gray-blue, transparent, sometimes irregular, $\frac{1}{2}$ to 1 mm. in diameter, under low power round slightly granular, pale yellow with entire edges. They increase in size, but slowly and are only slightly larger in three to four days. On hydrocele agar slants the colonies often coalesce to form a rather delicate homogeneous translucent growth. Hydrocele agar slants are better than plates for keeping the organisms alive.

*Ascites Fluid Agar.*—Ascites fluid may be added to agar just as hydrocele fluid and provides a favorable medium. The protein content of ascites fluid varies greatly and some samples work better than others. On good ascites fluid agar the colonies are often somewhat larger in forty-eight hours at 37° C., 2 to 3 mm., pale yellow-brown. They are larger than streptococcus or pneumococcus colonies, somewhat more opaque and often have a viscid sticky consistency.

*Blood agar* made from human blood or horse's blood is valuable for cultivation when other organisms are not present in the material examined. The colonies are small and fine, produce no hemolysis and in slants coalesce to a thin homogeneous layer.

Other solid media valuable for cultivation of the gonococcus are Vedder's starch agar, Wassermann's nutrose agar and serum gelatin (Leistikow, Loeffler[9]).

*Fluid media* are not satisfactory as a rule. The organism may be cultivated in Wertheim's broth containing 1 part blood-serum, ascites fluid, or pleural fluid to 2 to 3 parts meat infusion broth. Here it appears as a surface growth in twenty-four to forty-eight hours, a fine crumbly layer which on light shaking, or spontaneously, sinks to the bottom as fine floccules. It also grows in broth containing human or horse's blood. Slight acid is produced in fluid media containing dextrose.

No decomposition of urea.

**Oxygen Tension.**—The gonococcus grows aërobically and but little anaërobically. It grows well under partial oxygen tension. The atmospheric oxygen may be removed by suction, by gently heating the tubes or by the growth of some outspoken aërobe like *Bacillus subtilis*, an agar growth of which may be attached by a rubber tube to the tube containing the gonococcus culture. Swartz, Shohl, and Davis[10] have found that reduction of 10 per cent. of the atmospheric oxygen is sufficient to produce optimum growth, media with an initial reaction between pH 6.6 and pH 8.0 being most favorable. In media containing dextrose the acid end point is pH 5 to 6.

**Resistance** low. Gonococci die out in pus as soon as the pus dries, and survive but a few hours in moist pus, five hours according to Steinschneider.[11] Finger, Ghon, and Schlagenhaufer[12] report an exceptional instance when the organisms were cultivated from moist pus after seventy-two hours. In warm water (bath water) and urine they survive only one to two hours, rarely longer. In cultures they survive but a few days (eight to ten), rarely two to three weeks. Destroyed at 40° to 41° C. in a few hours. Remain alive at room temperature two to three days. Wassermann[13] reports that the organism could be cultivated from the valves of a heart kept in the ice-box twenty-four hours. Killed at 50° C. in ten minutes. Destroyed easily by disinfectants of which the most valuable are the silver salts. Killed in a few minutes by silver nitrate 1 : 1000, protargol 1.5 per cent., potassium permanganate 1:1000, resorcin 2 per cent., sublimate, with 1 to 10 NaCl, 1:10,000. (See Koch.[14])

**Pathogenic Action** on animals not marked. Wertheim observed that serum agar cultures introduced intraperitoneally produce peritonitis in white mice, and Nicolaysen[15] got the same result in guinea-pigs. At autopsy the peritoneum was free from exudate. Mice die from doses of $\frac{1}{2}$ to 1 c.c. serum-broth cultures introduced intraperitoneally. Rabbits also die, less promptly, from intravenous or intraperitoneal inoculation. Subcutaneous injection in rabbits produces infiltration which may lead to necrosis or abscess formation. Large doses

of broth cultures or emulsions of serum agar cultures (5 to 10 c.c.) kill guinea-pigs by intraperitoneal inoculation in twenty to thirty-six hours.

De Christmas,[16] Nicolaysen, and others believe that the gonococcus produces toxins, which are responsible for the lesions in animals. De Christmas regarded this toxin as liberated by the rapid death of the organism. Nicolaysen obtained his toxin from the dried bodies of the cocci. It was heat-resistant, destroyed at 100° C. only after a long time, and killed mice with a minimum lethal dose of 0.01 g.

**Lesions in Man.**—The gonococcus is found in a great variety of lesions in man. In the male it occurs in urethritis, epididymitis, orchitis, prostatitis, and occasionally in cystitis. In the female it is found in urethritis, vaginitis, endometritis, salpingitis, and occasionally in general peritonitis. It also occurs in the joints in arthritis, in endocarditis affecting the valves on the right side of the heart and in pericarditis. It is occasionally isolated from the blood (Thayer and Blumer,[17] Thayer and Lazear[18]), from abscesses in the muscles (Harris and Haskell[19]), from phlebitis (Heller[20]), from the spleen after gonococcus septicemia (Lenhartz[21]). It is a frequent cause of ophthalmia especially in children (ophthalmia neonatorum, blephritis). In children it may cause a proctitis sometimes epidemic in character, vulvovaginitis, etc. Pearce[22] and Torrey[23] regard the organisms found in children as of a different type.

1. Neisser: Centralbl. f. d. med. Wissensch., 1879, No. 28.
———: Deutsche med. Wchnschr., 1882, viii, 279.
2. Bumm: Arch. f. Gynæk., 1884, xxiii, 327.
———: Der Mikroorganismus der gonorrhoischen Schleimhauterkrankungen "Gonococcus Neisser." Wiesbaden, 1885.
3. Wertheim: Deutsche med. Wchnschr., 1891, xvii, 1351.
4. Neisser and Scholtz: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 1903, iii, 148.
5. Leistikow: Charité-Ann., 1882.
———: Berl. klin. Wchnschr., 1882, xix, 500.
6. Bockhart: Monatsychr. f. prakt. Dermat., 1886, v.
7. Haab: Die Mikrokokken der Blenorrh. Neonator. Wiesbaden, 1881.
8. Jadassohn: Deutsche med. Wchnschr., 1890, xvi, 542.
9. Löeffler: Deutsche med. Wchnschr., 1907, xxxiii, 169.
10. Swartz, Shohl, and Davis: Johns Hopkins Hosp. Bull., 1920, xxxi, 449.
11. Steinschneider: Berl. klin. Wchnschr., 1893, xxx, 696.
12. Finger, Ghon, and Schlagenhaufer: Arch. f. Dermat. u. Syph., 1894, xxviii, 3; 277.
13. Wassermann, M.: München. med. Wchnschr., 1901, xlviii, 298.
14. Koch, J.: Gonorrhea, *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann), 2. Aufl., 1912, iv, 655.
15. Nicolaysen: Centralbl. f. Bakteriol. [etc.], 1897, xxii, 305.
16. de Christmas: Ann. de l'Inst. Pasteur, 1897, ix, 609.
17. Thayer and Blumer: Johns Hopkins Hosp. Bull., 1896, vii, 57.
18. Thayer and Lazear: J. Exper. M., 1899, iv, 81.
19. Harris and Haskell: Johns Hopkins Hosp. Bull., 1904, xv, 395.
20. Heller: Berl. klin, Wchnschr., 1904, xli, 609
21. Lenhartz: München. med. Wchnschr., 1897, xliv, 1337.
22. Pearce: J. Exper. M., 1915, xxi, 289.
23. Torrey, J.: J. Med. Research, 1907, xvi (n. s. xi), 329.

## NEISSERIA INTRACELLULARIS OR MENINGITIDIS (Weichselbaum)

Observed microscopically in the spinal fluid in cases of sporadic meningitis by Marchiafava and Celli,[1] first cultivated and identified by Weichselbaum[2] whose work was confirmed by a number of observers all over the world; the work of Councilman, Mallory and Wright,[3] Flexner and Jobling,[4] Wollstein,[5] Elser and Huntoon[6] being especially important for America. Known also as *Micrococcus meningitidis intracellularis*, *Diplococcus meningitidis intracellularis*, and "meningococcus." This organism is now established as the etiological agent in practically all epidemic meningitis and in many sporadic cases.

**Morphology.**—Biscuit-shaped diplococcus arranged in pairs in both exudates and cultures, closely resembling the gonococcus. The individual cocci are flattened when in the diplococcus, spherical when free, sometimes in tetrads and short chains. They show great variations in size and intensity of staining, measuring usually 0.5 to 0.75 $\mu$ in diameter. In exudates they are frequently intracellular, especially in the spinal fluid and in nasal secretions. Kutscher[7] regards this as a true phagocytosis. Many of the organisms lie outside the cell, however.

Fig. 42.—Meningococcus from culture on blood agar twenty-two hours old, stained with Gram and carbolfuchsin.

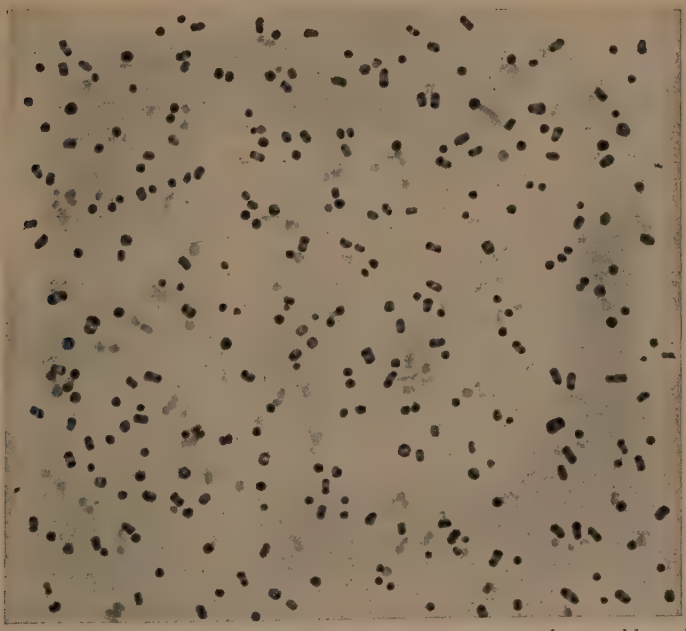


Fig. 43.—Meningococcus from culture on blood agar twenty-two hours old, stained with Gram and carbolfuchsin.

In cultures the organisms are typical only in very young cultures and even then show great variation in size and affinity for dyes. By the end of twenty-four hours the cultures contain, in addition to small and medium forms, involution forms, faintly stained individuals

and large cocci often deeply stained. In forty-eight hours the majority of the organisms have lost their typical morphology. Toluol added to cultures produces a degeneration of the organism (Flexner). Meningococci are dissolved like gonococci in sodium taurocholate (Ficker[8]).

**Staining Reactions.**—The meningococcus stains with the ordinary anilin dyes, but not so sharply as many other organisms. Organisms take gentian-violet badly and are best demonstrated by methylene-blue, safranin or by dilute carbolfuchsin. They decolorize regularly by Gram's method, usually in two to three minutes, and retain this property for long periods under artificial cultivation (Weichselbaum, Albrecht, and Ghon[9]). Metachromatic granules are frequently present in the organism and may be demonstrated by Neisser's stain or Loeffler's methylene-blue. Zinsser[10] states that Jenner's blood stain is most satisfactory for spinal fluid films.

**Cultivation.**—The meningococcus is cultivated with a certain degree of difficulty and shows great variations in its capacity to accustom itself to artificial media. It grows better on media containing human or animal protein. On ordinary agar it grows for one or two generations when the material used for transfer contains human protein. Probably the best medium is slightly alkaline meat infusion agar to which ascitic fluid, hydrocele fluid, or pleural exudate is added in the proportion of 1 to 3. Jochmann[11] has found that the addition of 1.5 per cent. glucose aids in the isolation because of the characteristic appearance of superficial colonies on this medium. Esch states that maltose serves the same purpose. Growth also good on serum agar or blood agar. Growth only above 25° C. Highest temperature permitting growth 42° C. (Albrecht and Ghon), 43° C. (v. Lingelsheim[12]). Strongly aërobic.

**Cultural Characters.**—*Ascites Agar Plates.*—Surface colonies appear usually in forty-eight hours, small, round, and regular, ½ to 1 mm. in diameter, somewhat more raised and opaque than streptococcus or pneumococcus colonies. Under low power granular, thicker in the center, thinner at the periphery, with entire edges. Deep colonies minute, under low power usually biconvex disks.

*Ascites Agar Slant.*—Growth appears in twenty to twenty-four hours at 37° C. as a gray, transparent, moist, glistening, homogeneous expansion which frequently has a slightly wavy edge along which isolated peripheral colonies develop. The growth is somewhat more abundant than that of the streptococcus or pneumococcus.

*Rabbit Blood Agar.*—Rather large opaque colonies in twenty-four hours, reaching their maximum in forty-eight to seventy-two hours, when they measure ½ to 2 mm. On slants the colonies coalesce to form a homogeneous layer. Blood not hemolyzed, retaining its natural color.

*Glucose or Maltose Ascites Agar.*—Colonies in twenty hours at 37° C. gray, transparent, moist, dull, slightly raised disks, 1 to 3 mm. in diameter. Under low power spherical, transparent, yellowish, homogeneous with smooth edges, sometimes slightly wavy. Colonies forty-eight hours old are 3 to 4 mm. in diameter with slightly raised brownish centers. Older colonies are darker in color. The colonies frequently have a crystalline appearance often seen also in *Micrococcus flavus* (Kutscher).

*Gelatin.*—Growth at room temperature slow and unsatisfactory in plates and stabs. No liquefaction. Growth in liquid gelatin at 37° C. as a surface film.

*Loeffler's Blood-serum.*—Small compact colonies like serum agar colonies, growth somewhat viscid, sometimes with a yellow or brown pigment.

*Glucose Agar.*—According to Kutscher ½ per cent. glucose agar made from Chapateaut peptone is a favorable medium. Large confluent colonies appear near the water of condensation. These yield good transfers, producing delicate transparent colonies, grayish-yellow in color. Glucose agar slants grayish-white to yellowish-gray. The culture mass when raised has a brownish color.

*Broth.*—No growth with some strains. Other strains grow as a diffuse turbidity or crumbly sediment, rarely as a fine fragile surface film. Growth better in ascites broth. No indol.

*Potato.*—Growth rare, sometimes a delicate succulent brownish expansion.

*Litmus Milk.*—Some multiplication without change in reaction.

*Fermentation tubes:*

*Dextrose.*—Growth in bowl and arm with precipitate. Reaction acid.

*Saccharose.*—Growth only in bowl with precipitate. No growth in closed arm. No acid.

*Lactose.*—Growth only in bowl with precipitate. No growth in closed arm. No acid.

According to v. Lingelsheim the meningococcus ferments maltose as well as dextrose, but not levulose, galactose, mannite, dulcite, saccharose, lactose, or inulin.

Other media useful for the cultivation of the meningococcus are Kutscher's placenta beef serum agar and Wassermann's nutrose agar.

*Growth at Partial Oxygen Tension.*—According to Wherry and Erwin,[13] and to Gates[14] the meningococcus grows better in an atmosphere in which some of the oxygen has been replaced by $CO^2$. By passing this gas into culture tubes of meningococcus and sealing them these

observers have been able to obtain large rapidly growing colonies. This has been confirmed by Zinsser.

**Resistance** to deleterious agents slight. Cultures die out rapidly on artificial media and often cannot be cultivated after the second or third generation. On solid media cultures kept from drying may survive fourteen to thirty days (Küster), rarely on serum media one hundred days (Shennan and Ritchie[15]).

Destroyed rapidly by disinfectants. Killed in one minute by 10 per cent. lysol, carbolic acid, creolin, chlorid of lime, and in bichlorid of mercury solution 1 : 1000. Destroyed in 2 to 5 creolin soap in one minute, in 5 per cent. hot soda solution at 52° C. in sixty minutes, in 2 per cent. at 63° C. in five minutes (Simon). Killed by formaldehyd steam in three and a half hours (Flügge). Destroyed by moist heat in five minutes at 50° C., in three minutes at 55° C., in one minute at 60° to 80° C., and in thirty seconds by boiling (Bettencourt and Franca[16]). According to v. Lingelsheim destroyed at 80° C. in two minutes, at 70° C. in five minutes, and at 50° C. in one hour. Dies out at room temperature and at the ice-box temperature in five days. In spinal fluid may survive on ice ten to eleven days (Trautmann and Fromme,[17] Stade[18]). Very sensitive to drying. Dried on glass plates organisms never survive more than twenty-four hours (Bettencourt and Franca). V. Lingelsheim has observed that meningococci suspended in salt solution and dried on coverslips or glass beads die the moment drying is complete. Destroyed by direct sunlight in two to six hours.

**Pathogenic Action.**—Meningococci are not pathogenic to ordinary laboratory animals on subcutaneous inoculation even in large doses. White mice sometimes die from intraperitoneal injection of 1 to 3 oesa of fresh cultures and young guinea-pigs can also occasionally be infected in the same way. Cultures vary greatly in activity, losing and assuming virulence from day to day from unknown causes (Kutscher). In animals dead of intraperitoneal inoculation there is a slight exudate on the peritoneum, usually viscid in character. Microscopically this shows many leukocytes full of degenerated cocci. Kolle and Wassermann[19] regard the infection as local only, but Elser and Huntoon found a septicemia in mice with meningococci in the heart's blood and in the organs. Rabbits are slightly sensitive, requiring large doses introduced intravenously for infection. Rats and pigeons are refractory.

In goats meningitis was produced by intraspinal injection by Councilman, Mallory and Wright. In apes v. Lingelsheim and Leuchs[20] produced the clinical symptoms and pathological lesions of meningitis by intraspinal injection of large doses. Flexner and Wollstein have also produced meningitis in apes by subdural inoculation.

A *toxin* in meningococcus cultures was first demonstrated by v. Lingelsheim and Leuchs, the filtrates of cultures three weeks old killing mice in doses of 0.25 to 0.5 c.c., guinea-pigs in doses of 3 c.c. by intraperitoneal injection. It was subsequently shown by Flexner[21] that filtrate from meningococcus emulsions kept under toluol are toxic to young guinea-pigs. It has also been found by Kraus and Doerr[22] that the toxin can be obtained by extracting twenty-four to forty-eight hour agar cultures with distilled water or with N/10 soda solution. It kills mice and guinea-pigs by intraperitoneal injection of 0.5 to 3 c.c. quantities. The animals died in eighteen to twenty-four hours, showing at autopsy a yellowish viscid exudate in the peritoneal cavity with a hyperemia of the intestinal serosa and the adrenals.

**Distribution.**—The meningococcus is present in the spinal fluid obtained by lumbar puncture (Councilman, Mallory and Wright), in the nasal secretions, and in metastatic abscesses. It has been found in the circulating blood by Dochez and Lamaire, Salamon, and many others. Present also in a great variety of locations in meningitis, such as arthritis (Lenhartz), bulbus disease (McKee), anterior chamber of eye (Tooke), herpes vesicles (v. Drigalski and Herford). It disappears from the nasal secretions in about three weeks (v. Lingelsheim), rarely persisting three months. Found in ulcerative pharyngitis by Reiche, in bronchial secretions by Hereford, in conjunctivitis by Davis, McKee, and Robinson, in conjunctivitis in an individual not suffering from meningitis by Brons, in a normal conjunctiva by McKee. At autopsy in meningitis cases it is found in the brain and spinal cord, in the spinal fluid, and in the meningeal pus. Not usually present in organs (Councilman, Mallory, and Wright). Found in spleen by v. Lingelsheim and Hereford. Found also in the metastatic inflammations of meningitis, pericarditis, endocarditis, plural exudates, areas of pneumonia, and bronchopneumonia.

Occasionally very rare complications of meningitis occur in which differential diagnosis between the meningococcus and the gonococcus is difficult and important. Thus Schottmüller[23] has described epididymitis as a complication of meningitis. Reuter[24] has observed purulent orchitis in meningitis and cultivated the meningococcus from the testicle. Infection of the seminal bladder has been reported by Pick.[25]

**Immune Reactions.**—Agglutinins have been found in the patient's blood by Bettencourt and Franca and by v. Lingelsheim, the highest titre being 1–100 or 1–200. Agglutinins are also present in the serum of immunized animals. Precipitates are produced when extracts of meningococci are mixed with specific sera. Immune sera have a bactericidal action (Flexner). Therapeutic sera have been prepared, especially by Kraus and Doerr, Flexner, Dopter, Flexner and Jobling, and Wassermann.

**Types of the Meningococcus.**—It was first shown by Dopter[26] in 1909 that some of the

meningococci obtained from cases in the vicinity of Paris could be distinguished from true meningococci by agglutination. These he called parameningococci. Since then the subject of meningococcus types has been investigated especially in England and America. Gordon and Murray,[27] studying the meningitis cases among British and Canadian troops, established four definite types on the basis of agglutination and absorption tests. These are distinguished as Type I, Type II, Type III, and Type IV. These types were confirmed by Tulloch[28] and by Fildes and Baker.[29] In this country Dopter's work was confirmed by Wollstein, who divided the parameningococcus into subdivisions, and by Flexner[30] and his associates at the Rockefeller Institute, who find two main types, normal meningococcus and parameningococcus, together with a number of intermediate strains. The normal meningococcus corresponds to Gordon's Type II, the parameningococcus to his Type I, while his Types III and IV embrace a considerable number of irregular or intermediate forms.

This typing is of importance chiefly in serum therapy since it is probably necessary to use a very large number of serological types in order to get efficacious antisera.

1. Marchiafava and Celli: Gaz. d. osp., 1884, No. 8, Jan. 27.
———: Centralbl. f. Bakteriol. [etc.], 1907, xliii, 141.
2. Weichselbaum: Fortschr. d. Med., 1887, v, 573; 620.
3. Councilman, Mallory, and Wright: Special Report Mass. State Board of Health, 1898.
4. Flexner and Jobling: J. Exper. M., 1908, x, 141; 690.
5. Wollstein: J. Exper. M., 1909, xi, 579.
6. Elser and Huntoon: J. Med. Research, 1909, xx (n. s. xv), 371.
7. Kutscher: Uebertragbare Genickstarre. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl., 1912, iv, 589.
8. Ficker: Arch. f. Hyg., 1908, lxviii, 1.
9. Albrecht and Ghon: Wien. klin. Wchnschr., 1901, xiv, 984.
10. Zinsser: Textbook of Bacteriology. 1922, p. 524.
11. Jochmann: Deutsche med. Wchnschr., 1906, xxxii, 788.
12. v. Lingelsheim: Klin. Jahrb., 1906, xv, 373.
13. Wherry and Erwin: J. Infect. Dis., 1918, xxii, 194.
14. Gates: J. Exper. M., 1919, xxix, 325.
15. Shennan and Ritchie: J. Path. and Bacteriol., 1908, xii, 456.
16. Bettencourt and Franca: Ztschr. f. Hyg., 1904, xlvi, 463.
17. Trautmann and Fromme: München. med. Wchnschr., 1908, lv, 1, 791.
18. Stade: Hyg. Rundschau, 1908, xviii, 519.
19. Kolle and Wassermann: Klin. Jahrb., 1906, xv, 507.
20. v. Lingelsheim and Leuchs: Klin. Jahrb., 1906, xv, 489.
21. Flexner: J. Exper. M., 1907, ix, 105; 142; 168.
22. Kraus and Doerr: Wien. klin. Wchnschr., 1908, xxi, 12.
23. Schottmüller: München. med. Wchnschr., 1905, lii, 2, 1617; 1729.
24. Reutter: Biologische Abteilung des arztl. Vereins zu Hamburg, 6, vi, 1905.
———: (Ref. München. med. Wchnschr., 1905, lii, 2, 1617.)
25. Pick: Berl. klin. Wchnschr., 1907, xliv, 994.
26. Dopter: Compt. rend. Soc. de biol., 1909, lxvii, 74.
27. Gordon and Murray: J. Roy. Army Med. Corps, 1915, xxv, 411.
28. Tulloch: J. Roy. Army Med. Corps, 1918, xxx, 115.
29. Fildes and Baker: Brit. Med. Res. Com. Spec. Rep., Ser. No. 14, London, 1918.
30. Flexner: Monog. Rockefeller Inst. M. Research, 1917.

### NEISSERIA CATARRHALIS (Pfeiffer)

Originally described as *Micrococcus catarrhalis* by Pfeiffer[1] in the nasal secretions in simple catarrhal conditions of the upper respiratory tract and studied more extensively by Ghon and Pfeiffer.[2]

A Gram-negative coccus a little larger than the meningococcus and less like a diplococcus, 0.6 to 0.8 $\mu$ in size, occasionally intracellular in exudates. Cultivated from the first generation on ordinary media. On ascites agar it produces whitish, somewhat hard, compact colonies, with an uneven dry surface, somewhat smaller than meningococcus colonies of the same age. Under low power they are brown, granular, and irregular, with jagged edges which show a lighter zone with radial striations. Colonies dry and viscid and can be lifted "in toto" with a platinum needle, often breaking up into separate masses. On ascites agar it forms a thick white expansion which can be transferred after eight days. No action on carbohydrates. Spontaneously agglutinated in salt solution. Grows at temperatures below 20° C. Little pathogenic action on animals.

1. See Flügge: Die Mikroorganismen. 3. Aufl. 1896, ii, 154.
2. Ghon and Pfeiffer: Zeitschr. f. klin. Med., 1902, xliv, 262.

### Neisseria crassa (Kutscher)

Described as *Diplococcus crassus* by Kutscher[1] and as identical with the Jaeger variety of meningococcus.[2] Found in the normal pharynx, but may wander into the meninges in meningitis due to other organisms. Found also by v. Lingelsheim in tuberculous meningitis and in meningitis after trauma.

Diplococci and tetrads somewhat larger than the meningococcus. Produces involution forms readily. In old cultures more spherical and like a staphylococcus. Majority of cells Gram-positive, a few always Gram-negative.

Grows on ordinary agar in a delicate expansion like that of a streptococcus. Ascites agar growth less abundant than that of the meningococcus, whitish-gray. Colonies on ascites agar smaller and more compact than those of the meningococcus, grayish-brown by transmitted light. Grows at 20° C.

More resistant than the meningococcus.

Ferments dextrose, maltose, levulose, saccharose, lactose, and galactose.

1. Kutscher: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl., 1912, iv, 589.
2. Jaeger: Ztschr. f. Hyg., 1895, xix, 351.

### Neisseria sicca (v. Lingelsheim)

Reported as *Micrococcus pharyngis siccus* by v. Lingelsheim[1] from the normal and inflamed respiratory mucosa.

Gram-negative cocci, 0.6 to 0.8 $\mu$ in diameter, occurring singly and in pairs like meningococci.

Grows as an aërobe at 37° C. on ordinary media.

Colonies on blood agar grayish, dry, crenated, easily broken.

Ascites agar colonies small, firm. adherent, crenated, and corrugated.

Acid in dextrose, maltose, levulose, and saccharose (Elser and Huntoon). Spontaneously agglutinated in salt solution.

Kidney infections from this organism have been reported by Kretschmer and Hufnagel,[2] and a case of acute vegetative endocarditis by Schultz.[3]

1. v. Lingelsheim: Klin. Jahrb., 1905, xv. Ztschr. f. Hyg., 1908, lix, 457.
2. Kretschmer and Hufnagel: J. Am. M. Ass., 1924, lxxxii, 1850.
3. Schultz: J. Am. M. Ass., 1918, lxxi, 1739.

### Neisseria flava (v. Lingelsheim)

Described as *Micrococcus flavus* by v. Lingelsheim as a common inhabitant of the normal throat. Classified as Chromogenic Group II by Elser and Huntoon.

Gram-negative cocci about 0.6 to 0.8 $\mu$ in diameter, occurring singly and in pairs, and much resembling meningococci.

Grows best as an aërobe from 22° to 37° C.

Colonies on dextrose and ascites agar small, circular, slightly raised, greenish-gray by reflected light, and greenish-yellow by transmitted light. Growth not adherent to the medium. Surface colonies smooth, with numerous coarse, crumbly granules in the center. Margins entire or rarely slightly irregular. Produces a pale yellow pigment.

Acid in dextrose, maltose, and levulose.

Elser and Huntoon: J. Med. Research, 1909, xx (n. s. xv), 371.

### Neisseria perflava Bergey

Described by Elser and Huntoon[1] as Chromogenic Group I of the Gram-negative cocci from the throat. Named by Bergey.[2]

Gram-negative spherical organisms 0.6 to 0.8 $\mu$ in size, occurring singly and in pairs. Grows best aërobically at 37° C.

Colonies on dextrose agar and ascites agar small, circular, slightly raised, greenish-gray

by reflected light, greenish-yellow and semiopaque by transmitted light. Growth adherent to the medium. Surface smooth and glistening.

Acid in dextrose, maltose, levulose, and saccharose.

1. Elser and Huntoon: J. Med. Research, 1909, xx (n. s. xv), 371.
2. Bergey: p. 43.

### NEISSERIA SUBFLAVA Bergey

Described by Elser and Huntoon[1] as Chromogenic Group III of the Gram-negative cocci in the respiratory mucous membrane. Named by Bergey.[2]

Gram-negative spherical organisms 0.6 to 0.8 $\mu$ in size, occurring singly and in pairs.

Grows best as an aërobe at 37° C.

Colonies on dextrose agar small, circular, slightly raised, greenish-yellow.

Acid in dextrose and maltose.

1. Elser and Huntoon: J. Med. Research, 1909, xx (n. s. xv), 371.
2. Bergey: p. 44.

### NEISSERIA MUCOSA (Elser and Huntoon)

Described originally as *Diplococcus mucosus* by Elser and Huntoon from the secretions of the nose and throat.

**Morphology.**—Gram-negative diplococci, somewhat resembling the meningococcus, but provided with capsules easily stained by Hiss's method.

**Cultural Characters.**—Colonies on ascites agar plates somewhat like those of the meningococcus. After twenty-four hours' incubation they are 3 to 4 mm. in size with a smooth glistening surface. They are more succulent and mucinous in appearance. Luxuriant growth on serum-free glucose agar, tending to become confluent. Good growth at room temperature.

Elser and Huntoon: J. Med. Research, 1909, xx (n. s. xv) 371.

### DIPLOCOCCUS INTRACELLULARIS EQUI Johne

Found by Johne[1] in Borna's disease of horses (acute encephalitis). Same as Ostertag's Bornastreptococcus from the same disease.[2] Not yet proven to be the cause of the condition (Joest[3]).

An aërobic non-motile Gram-negative micrococcus. No liquefaction of gelatin or blood-serum. Growth in broth and in condensation water of solid media as a diffuse turbidity, clearing after eight days, with an abundant gray-white sand-like sediment. It grows at first but slightly on artificial media, later more abundantly. Lohr[4] reports the potato growth as delicate, glistening, watery-gray. No fermentation of glucose, levulose, galactose, dulcit, saccharose, lactose, or maltose. No indol, $H_2S$, or pigment. Petruschky's milk-whey reddish or pale blue. No change in Loeffler's green. No growth on v. Drigalski or on Endo.

Growth at 37° to 38° C., better at 41° to 42° C. No growth at 45° C. No growth below 12° C.

Dies rapidly in distilled water. Grows in water containing nitrogenous pollution. Killed rapidly by drying. Lives in moist substrata four weeks.

1. Johne: Deutsche Ztschr. f. Thiermed., 1897, xxii, 369.
2. Ostertag: Berl. tierärztl. Wchnschr., 1900, No. 37, 433.
3. Joest: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, vi, 251.
4. Lohr: Beiträge zur Bakteriologie der Gehirnruckenmarksseuche der Pferde. Inaug. Dissert., Leipzig, 1910.

### DIPLOCOCCUS ALBUS TARDISSIMUS Eisenberg

Found in secretions from the vagina and cervix, especially in the vaginal secretions of puerperal women. Described originally by Bumm.[1] Named by Eisenberg.[2]

**Morphology.**—Diplococci like gonococci, two biscuit-shaped halves separated by a narrow cleft. Average diameter from pole to pole 1.25 $\mu$.

**Biological Characters.**—Grows very slowly at the room temperature in the usual media.
*Agar Slant.*—Slow development as a thin, moist, grayish-white layer with dentate margins.
*Gelatin Plates.*—Extremely small punctiform colonies, under low power spherical, opaque, and brownish-green. At the end of two weeks they may have a diameter of 2 mm.
*Gelatin Stab.*—Small, isolated, grayish-white colonies developing after several days along the line of inoculation. Surface growth slow as a thin, whitish, stearin-like layer with irregular dentate margins.
**Remarks.**—Of interest because of its resemblance to the gonococcus.

1. Bumm: Der Mikroorganismus der gonorrhoischen Schleimhauterkrankung. 2. Aufl. Wiesbaden, 1887.
2. Eisenberg: Bakteriologische Diagnostik. 3. Aufl. 1891, p. 58.

## Tribe B. Streptococceæ Trevisan, emended Com.

Parasites (thriving only or best on or in the animal body) except genus *Leuconostoc*. Grow well under anaërobic conditions. Many forms grow with difficulty on serum-free media, none very abundantly. Planes of fission usually parallel, producing pairs or short or long chains, never packets. Generally stain by Gram. Produce acid, but no gas in glucose and generally in lactose broth. Pigment, if any, white or orange.

### GENUS 2. DIPLOCOCCUS Weichselbaum, emended Com.

Parasites, growing poorly, or not at all, an artificial media. Cells usually in pairs of somewhat elongated cells, often capsulated, sometimes in chains. *Gram-positive.* Fermentative powers high, most strains forming acid in glucose, lactose, sucrose, and inulin.

Type species, *Diplococcus pneumoniæ* Weichselbaum.
" *mucosus* (Schottmüller).
" *lanceolatus ovum* Gärtner.
" *griseus non-liquefaciens* Tissier and Martelly.
" *tetragenus* (Gaffky).
" *ovalis* (Escherich)
" *griseus liquefaciens* Tissier.
" *fœtidus* (Klamman).

### Diplococcus pneumoniæ Weichselbaum

The etiological agent in true lobar pneumonia, in many cases of bronchopneumonia and in various complications of these diseases, usually known as the "pneumococcus," was first definitely described by Pasteur, Chamberland, and Roux[1] although in all probability it was seen earlier by Eberth[2] and by Koch.[3] Pasteur obtained it by injecting rabbits with saliva and called it the "microbe of rabbit septicemia." The same organism was later obtained by Sternberg.[4] Subsequently organisms were cultivated from pneumonia by Friedländer[5] and for a brief period regarded as the causative factor in the disease. The confusion was cleared up by Fraenkel[6] and Weichselbaum[7] working independently about the same time. These authors cultivated the organism of sputum septicemia from the lungs and pleura of cases of lobar pneumonia and from some of the other organs and brought forward considerable evidence that the organisms present in the normal mouth and the organisms found in the lesions of pneumonia were identical. The true relation of these micro-organisms has been worked out only within the past few years, chiefly by the investigations of Neufeld and Haendel,[8] Cole,[9] Dochez and Gillespie[10] and Lister.[11]

**Morphology.**—Small, round, or oval organisms in the sputum, blood or tissues, usually arranged in pairs end-to-end, each pair being enclosed in a delicate capsule. The individual

Fig. 44.—Pneumococcus Type I, from blood agar culture twenty-four hours old, stained by Gram.

Fig. 45.—Pneumococcus Type I, from forty-eight-hour blood-serum culture, stained by Huntoon.

cells are frequently slightly elongated and oval or lance-shaped, measuring 0.5 by 0.7 $\mu$. The pairs are often joined end-to-end, shorter or longer chains being produced. The capsular substance may coalesce and surround a short chain of organisms. In fluid exudates chains of pneumococci are common. In cultures the typical morphology is by no means so common.

The capsules are demonstrated with greater difficulty, the capsular substance may be much less in quantity and the individual cells appear round or slightly oval rather than elongated. The organisms are frequently grouped in smaller or larger bunches. In consequence films from cultures often show organisms which look like staphylococci. Capsules can usually be demonstrated, however, if films are made in serum or some other albuminous solution. They are demonstrated more easily from cultures in milk, blood agar, blood-serum, serum or ascites fluid agar or sterile pneumonic sputum than from cultures on agar or in broth. In old cultures the cells are often longer, almost rod-shaped, and rarely chains are present in considerable abundance. Occasionally the cells are enlarged, round, oval or irregular in shape. In old broth cultures the sediment may consist chiefly of chains. No spores. Non-motile. No flagella.

**Staining Reactions.**—The pneumococcus stains easily by the usual anilin dyes, gentian-violet, methylene-blue, safranin, or dilute carbolfuchsin and is strongly Gram-positive. The capsules are lost by these stains and require special methods for their demonstration of which a great number have been devised. They may be brought out by staining the organisms in anilin water gentian-violet, washing in alcohol, followed by eosin (Friedländer), anilin water fuchsin and methylene-blue (Wolf[12]) or by the use of osmic acid vapor followed by

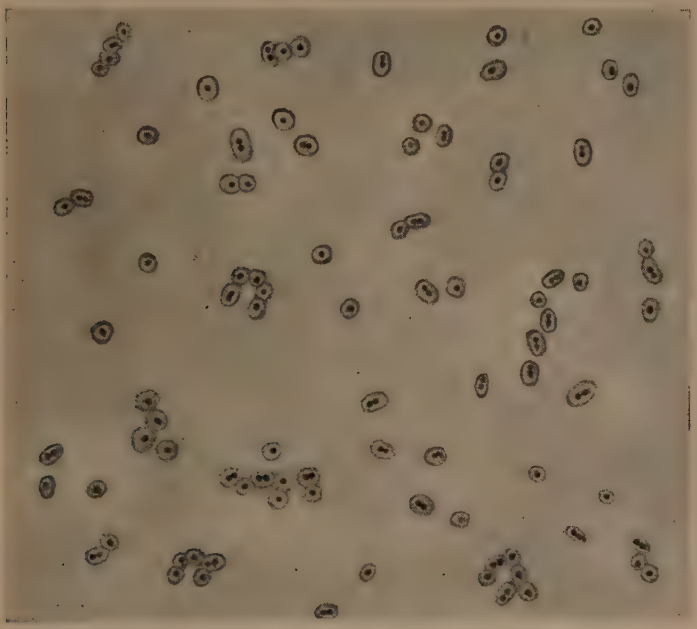


Fig. 46.—Pneumococcus Type II, from peritoneal exudate in mouse, stained by Huntoon.

Loeffler's methylene-blue (Heim[13]). A number of methods of staining capsules have been devised in America of which the best are possibly those of Welch,[14] Hiss,[15] Wadsworth,[16] Rosenow,[17] Buerger,[18] and Huntoon.[19] The chemical composition of the capsule is not definitely ascertained. According to Friedländer the capsule is made up of mucinous substance, according to Guarnieri it is made up of albuminoids. In the tissues the pneumococcus is best demonstrated by the methods of Gram, Weigert, Paltauf's modification of Gram's method, or by Goodpasture's stain.

**Cultivation.**—The pneumococcus can be cultivated under aërobic and anaërobic conditions without great difficulty, but requires considerable care in its original isolation. It may be grown directly from infected lung tissue or from the blood by transferring considerable material to such media as blood agar or serum agar. It is more difficult to get the organisms from the sputum because of the admixture of other bacteria, and may be isolated most easily by the inoculation of mice. The animals die in a few hours; the organisms are present in great numbers in the heart's blood, and cultures may easily be obtained on ordinary media. Growth at temperatures between 25° and 42° C. Optimum temperature 37° C. Old cultures accustomed to artificial conditions grow at lower temperatures, 18° to 25° C. The media should have a slightly alkaline reaction, best obtained by adding 10 to 12 c.c. normal sodium hydrate to each liter.

**Cultural Reactions.**—*Plain Agar Plates.*—Colonies develop slowly, usually appearing at the end of forty-eight hours. The superficial colonies are very small, isolated, round, water-clear, like dew-drops, measuring ½ to 1 mm. They resemble colonies of the streptococcus, but are more transparent. They are sometimes flatter and more disk-shaped with a slight central retraction. Under low power they show a dark central nucleus which may be slightly granular and a lighter peripheral zone. The edge of the colony is usually regular, but may be slightly indented. Deep colonies are very small, barely visible to the naked eye, under low power yellow-brown, oval, lens- or whetstone-shaped, often quite characteristic. The colonies are slightly larger in three to four days, but seldom are more than 1½ mm. in diameter.

*Plain Agar Slant.*—Very delicate barely visible scanty growth in forty-eight hours, usually made up of small dew-drop colonies.

*Plain Agar Stab.*—Delicate growth along the line of the stab in two to three days, very slight surface expansion at the point of puncture.

*Blood Agar* (rabbit's blood or human blood).—Growth on slants more vigorous than on plain agar, usually with a greenish-black discoloration of the blood pigment. Colonies on blood agar are surrounded by a greenish zone and occasionally show a delicate area of hemolysis inside this (Cole). After four to five days the colonies are much larger, thick and blackish, not to be mistaken for streptococcus colonies.

*Glucose Litmus Agar.*—Surface colonies somewhat larger than plain agar colonies in forty-eight hours, round, regular, and uniform. Under low power they are slightly granular, round and regular, with entire edges. Deep colonies punctiform, under low power oval and wedge-shaped. In two to three days the organism produces considerable acidity so that each colony is surrounded by a zone of acid agar. The acidity may be so great as to turn the entire plate acid.

*Glucose Litmus Agar Stab.*—Uniform growth along line of inoculation with slight surface expansion in forty-eight hours. The agar is gradually turned acid.

*Loeffler's Blood-serum.*—Rather delicate dry granular expansion in two to three days.

*Serum Agar* (1 part human serum to 2 parts agar).—Good growth, colonies large and viscous. Ascites fluid or hydrocele fluid may be used instead of the serum.

*Gelatin.*—No growth on gelatin at 20° C., very slight growth at 24° to 25° C. with freshly isolated strains. Old strains grow on gelatin at 18° to 20° C. as delicate white colonies without liquefaction.

*Potato.*—Usually no growth. Occasionally a thin barely visible growth almost the color of the potato which microscopically shows many pneumococci (Ortmann).

*Broth.*—Uniform turbidity and fine flocculent sediment in twenty-four to forty-eight hours. The broth usually clears in a few days, and this may be due to partial autolysis (Kruse and Pansini,[20] Buerger). In old cultures and occasionally in fresh cultures the pneumococcus may grow from the start as a fairly heavy sediment.

*Blood Broth* (rabbit or human blood).—Growth somewhat more marked than in plain broth. No hemolysis of the blood usually, but a greenish discoloration from methemoglobin.

*Peptone.*—Very slight turbidity and sediment. Growth never abundant.

*Fluid Blood-serum* (human or animal).—Usually a good growth with turbidity and abundant sediment.

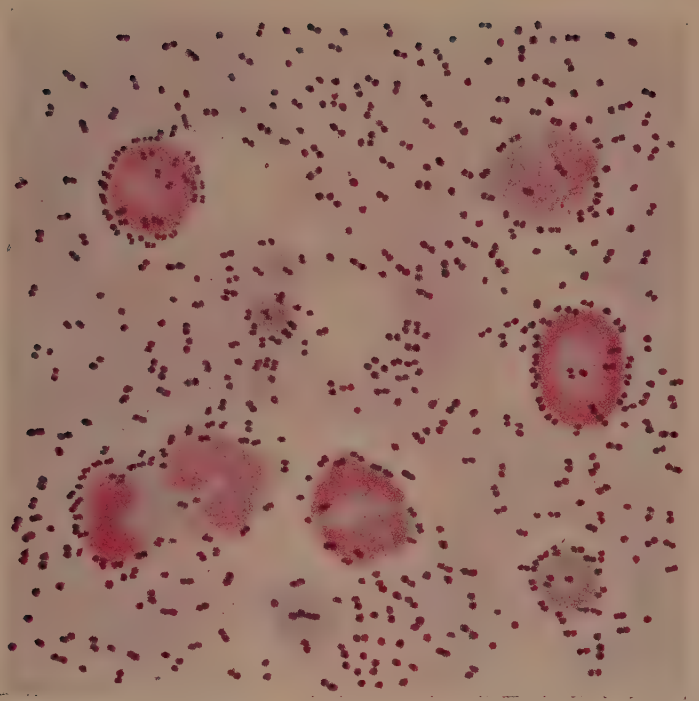
*Litmus Milk.*—Fairly rapid development with an acid reaction and a coagulation in two to three days. According to Kruse and Pansini this coagulation is not always evident.

*Litmus Whey.*—Fairly vigorous growth with acid production.

*Glucose Broth.*—Abundant development with the production of considerable amounts of acid, this acidity being great enough to kill the organism. In the Smith fermentation tube growth takes place in both the bowl and closed arm. Nissen[21] and Kruse and Pansini regard the acid as chiefly lactic acid, but Würtz and Mosny[22] state that formic acid is also produced. By the addition of potassium carbonate (Würtz and Mosny) or calcium carbonate in the shape of pieces of powdered marble (Hiss) the acids are neutralized and the organism may be kept alive for longer periods. Acidity also from lactose, not from saccharose.

*Hiss Inulin Serum Water.*—The pneumococcus multiplies well in this medium, producing acid from the inulin and coagulating the serum. Streptococci, as a rule, fail to act on inulin. Recent investigations indicate that not all pneumococci produce acid from inulin and a few streptococci do produce it (Holman). Other media suitable for the pneumococcus but less popular are Guarnieri's gelatin agar, brain broth or brain agar, fresh hen's egg, and hemoglobin agar.

*Bile Salts.*—Pneumococci are rapidly killed and dissolved by bile salts (Neufeld). A few drops of rabbit's bile converts a pneumococcus broth culture to a clear transparent fluid even when mixed with two hundred or three hundred times its volume of fluid. Microscopically no pneumococci are left visible. Human bile has the same effect. The action of the bile is due to bile salts. Glycocol and taurin have no effect, but glycocol taurocholate and taurocholic acid exhibit this solvent action. The membrane of the pneumococcus and the capsule swell and disintegrate in bile so that they no longer protect the organism. Bile is hemolytic and dissolves other body cells as well as certain protozoa, but hemolytic agents in general do not



Pneumococcus Type II, from peritoneal exudate in mouse, stained by Gram.

dissolve pneumococci. Pneumococci killed by heat are not dissolved and old cultures and partially dead cultures are not completely dissolved. Neufeld and Haendel have found that avirulent strains of pneumococcus are not dissolved by bile. Old agar cultures become avirulent, the colonies are somewhat opaque and coherent and tend to grow at room temperatures. Such cultures are not dissolved by bile. Bile has no action on streptococci and but little action on meningococci (Ficker).

**Pathogenic Action.**—The pneumococcus is pathogenic to small animals, especially to mice and rabbits, for which it is virulent to about the same degree. Freshly isolated pneumococci kill mice and rabbits by subcutaneous, intraperitoneal, and intravenous injection of 0.0001 to 0.0000001 c.c. of a fluid culture in two to three days. The animals develop a true septicemia with the organisms distributed everywhere in the blood and organs, especially in the spleen. In rabbits subcutaneous injection produces at times an edema and a swelling at the site of inoculation. Injection into the ear gives rise to characteristic erysipelas-like local inflammation (A. Fraenkel, Neufeld). Virulent cultures also produce conjunctival and corneal infections leading to septicemia. Old strains of lessened virulence, injected subcutaneously, sometimes produce in rabbits local edema, abscesses, and inflammations of the joints and serous membranes such as the pericardium, instead of the characteristic septicemia.

Rarely mice are more sensitive than rabbits (Gamaléia[23]), and Ungermann[24] has described an exceptional strain of pneumococcus highly virulent for mice and not virulent for rabbits in doses of 1 c.c.

Guinea-pigs are sensitive to the pneumococcus, but less regularly so than mice and rabbits. A pleuropneumonia has been produced by intrapleural inoculation by Monti, by direct injection into the lungs by Neufeld and Engwer.[25]

Dogs are refractory to intravenous and intraperitoneal inoculation, but are sensitive to subcutaneous injection (Kruse and Pansini) and to subdural inoculation (Monti). Infection can also be produced occasionally in pigeons and young kittens. Cats and sheep are also sensitive to the pneumococcus. Chickens and usually pigeons are resistant.

Rats are sensitive. Lamar[26] and Neufeld and Haendel have produced a septicemia fatal in two days by subcutaneous injection of 0.000001 c.c. of a virulent strain.

With refractory animals local inflammations are more common than septicemias.

**Production of True Lobar Pneumonia.**—While many animals are sensitive to pneumococcus infection the lesions found do not resemble true lobar pneumonia in man. Thus A. Fraenkel and Gamaléia were not able to produce a true pneumonia in rabbits by tracheal, pleural, and intrapulmonary injection. With cultures of lessened virulence Fraenkel and Guarnieri obtained some evidences of hepatization of the lung, and Kruse and Pansini produced a purulent pleurisy with partial hepatization of the lungs in dogs by tracheal and intrapulmonary inoculation of 1 to 2 c.c. Wadsworth[27] found pneumonic lesions in rabbits almost identical with those found in man after partial immunization and intratracheal injection of pneumococci varying in virulence. Somewhat later Lamar and Meltzer[28] found a true lobar pneumonia in dogs after intratracheal insufflation of large quantities, this being confirmed by Winternitz and Hirschfelder.[29] Finally, a true lobar pneumonia has been produced in monkeys (*Macacus rhesus*) by Cecil and Blake[30] by the injection of virulent pneumococci in doses of 0.1 to 0.2 c.c. directly into the trachea.

The *virulence* of pneumococcus cultures varies greatly. It is lost rapidly on ordinary agar cultures and is shown usually only in fluid cultures (serum-broth). After long passage through mice it may be greatly reduced for rabbits. The virulence for rabbits may be restored by passage through rabbits (Neufeld and Haendel, Roemer[31]). In general, the virulence is lost rapidly under artificial cultivation and can be maintained best by animal passage. In blood and in the tissues of infected animals the virulence may be maintained by keeping the material in the desiccator at low temperatures (Neufeld and Haendel). Foà[32] found that the virulence of cultures may be maintained sixty days by cultivating the pneumococci from rabbits dead of septicemia for twenty-four hours at 37° C. and then putting the cultures in the cold and dark. On coagulated blood Kindberg found them virulent for four and a half months. Sclavo[33] states that the virulence has been maintained for sixty-seven days by keeping the spleen of infected animals in glycerin. Suetscher[34] has found that strains of pneumococcus isolated during the progress of a case of pneumonia all possess the same virulence. The virulence of pneumococci from corneal ulcers is like that of pneumococci from other infections.

**Lesions in Man.**—The pneumococcus is present in a large proportion of the cases of true lobar pneumonia in man and in many cases of bronchopneumonia. In some cases of lobar pneumonia it may be obtained from the blood-stream. The same type of pneumococcus is usually present in the sputum as in the lung lesions. It is found in many of the complications of pneumonia, such as pleurisy, empyema, otitis media, mastoiditis, meningitis, endocarditis, pericarditis, and arthritis. Peritonitis is occasionally due to pneumococcus, especially in young children, and severe eye infections accompanied by ulceration of the cornea are frequently traced to the pneumococcus. Pneumococcus infections may likewise occur in the bones and in the kidneys. Pneumococcus peritonitis has also been described in young children, with the vulva as the portal of entry.

**Resistance.**—In cultures the pneumococcus has little viability, living but a few days in solid media. The organisms survive longer on blood or serum media than on ordinary media and better at low temperatures than at high temperatures. Deep agar and gelatin cultures may survive a long time in the ice-box. They are destroyed easily by heat at 52° C. in ten minutes (Sternberg). They are killed by weak solutions of ordinary disinfectants, bichlorid of mercury, carbolic acid, etc. According to Wadsworth they are destroyed most rapidly in exudates by 20 per cent. alcohol, stronger disinfectants being less efficient. In the blood and organs of infected animals they remain viable for a long time. Neufeld states that blood in thick layers or pieces of infected tissue like the spleen from animals dead of septicemia may be kept in open Petri plates in a desiccator for long periods and still contain viable pneumococci, which may be obtained by injection into rabbits or mice subcutaneously or intraperitoneally. The original virulence is maintained by this method sometimes for a year. In blood pneumococci resist drying, sunlight, and putrefactive bacteria. Guarnieri finds that pneumococci in blood dried rapidly on bristle brushes remain alive a month. In sputum the pneumococcus survives for variable periods. According to Bordoni-Uffreduzzi[35] the pneumococcus in dried sputum remains alive in diffuse daylight for fourteen to fifty-five days. In sputum mixed with sterile dust and then dried Germano[36] found the organisms alive after one hundred and twenty to one hundred and fifty days. Netter found viable pneumococci in the dust of hospitals after twenty-seven days. Pneumococci in sputum dried on pieces of linen cloth survive in diffuse daylight seventy to eighty days (Ottolenghi[37]). Pneumococci in blood or pus dried on silk threads and kept in a dessicator have been found alive after a year by Heim. Bordoni-Uffreduzzi thinks that the albuminous and viscid substances in the sputum protect the organism. Neufeld and Haendel believe that in drying in a desiccator a film is formed about the organisms which protects them from light and air and keeps the interior moist. When this protective layer is lacking the pneumococci die like ordinary bacteria.

**Pneumococcus Types.**—Following the observation of Neufeld and Haendel in 1910 that pneumococci vary greatly in virulence and serological reactions, it was shown by Dochez and Gillespie in Cole's laboratory that certain well-marked types may be made out among the pneumococci. These types may be exactly differentiated by serological reactions and protective tests. We now recognize four different pneumococcus types by immunological reactions.

Type I.—Characteristic pneumococci all agglutinated by the same serum and showing the same protective tests.

Type II.—Similar pneumococci agglutinated by another serum and showing protective tests by it.

Type III.—*Pneumococcus mucosus capsulatus*, originally described as *Streptococcus mucosus capsulatus*.

Type IV.—Heterogeneous strains of pneumococcus, usually of low virulence and present in the normal mouth.

Type II has now been split up into subgroups:

IIa, IIb, and IIc by Avery[38] and Omstead[39] has shown that the organisms put in Type IV are many of them related in small groups.

Similar types of pneumococci have been found by Lister in South Africa. They are designated A. B. and C. Type C corresponds to the American Type I, Type B to the American Type II, while Type A apparently does not occur in America.

Pneumonia in man is due usually to members of Type I and Type II, that due to Type III being much less common. Pneumonia may be produced by members of Type IV, the organisms found in the normal mouth, but this type of pneumonia is less apt to be fatal. Recovery is rare in pneumonia due to Type III. Type I pneumonia is usually associated with a higher mortality than Type II. Dochez and Avery[40] found that over 80 per cent. of the pneumonias in and about New York City belong to Types I, II and III, while over 50 per cent. of the ordinary mouth pneumococci belong to Type IV. Observations in other cities in America have been found in practical accord with Dochez and Avery's figures for New York. Similar typing of the pneumococcus has been carried out by Blake by means of the precipitin test.

*Soluble Products.*—Attempts to obtain toxic substances from pneumococcus cultures have not proved eminently successful. G. and F. Klemperer[41] found that filtrates from pneumococcus cultures produce fever and eventually death in young rabbits by the administration of large doses. MacFadyen[42] obtained a poisonous substance by freezing the organisms and grinding them up in 1 per cent. potassium hydrate. This killed guinea-pigs in doses of 0.05 c.c. Somewhat similar toxic products have been found by Wadsworth.[43] It has been shown by Cole[44] that the pneumococcus cells contain a hemolytic substance. This is liberated when the pneumococcus is dissolved in bile salts. It is destroyed by heat and by tryptic digestion. Cole regards this as an intracellular hematoxin and differentiates it from the substance that changes hemoglobin into methemoglobin.

**Immunity Reactions.**—Large animals may be actively immunized to the pneumococcus and their serum has been employed on a considerable scale for passive immunization during the course of pneumonia. The pneumococcus present in the patient's sputum must be accurately typed and that serum administered which has come from an animal immunized with

the same type. Type I and Type II sera have been especially employed in the treatment of pneumonia under Cole at the Rockefeller Hospital in New York. No satisfactory serum has thus far been found for Type III. The most favorable results have been obtained with Type I serum. Vaccination against pneumonia has been employed chiefly by Wright in England, by Lister in South Africa, and by Cole and Austin in America. The vaccines are usually salt solution suspensions of pneumococci killed at 56° C. and preserved with 0.3 per cent. trikresol. The reports thus far published are not conclusive.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7. Weichselbaum: Med. Jahrb., 1886, n. F., i, 483.
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11. Lister: Pub. South African Inst. M. Research, 1916, No. 8.
12. Wolf: Wien. med. Bl., 1887, Nos. 10–14.
13. Heim: Ztschr. f. Hyg., 1905, l, 123.
14. Welch: J. Am. M. Ass., 1904, iv, 23.
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16. Wadsworth: J. Med. Research, 1903–04, x (n. s. v), 228.
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18. Buerger: Med. News, 1904, lxxxv, 1117.
19. Huntoon: J. Bact., 1917, ii, 241.
20. Kruse and Pansini: Ztschr. f. Hyg., 1892, xi, 279.
21. Nissen: Fortschr. d. Med., 1891, ix, 661.
22. Würtz and Mosny: Semaine méd., 1894,
23. Gamaléia: Ann. de l'Inst. Pasteur, 1888, xii, 440.
24. Ungerman: See Neufeld and Haendel, *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1912, iv, 513.
25. Neufeld and Engwer: Berl. klin. Wchnschr., 1912, xlix, 2, 2381.
26. Lamar: J Exper. M., 1910, xiii, 1. *Ibid.*, 1911, xiv, 256.
27. Wadsworth: Am. J. M. Sc., 1904, cxxvii, 851.
28. Lamar-Meltzer: J. Exper. M., 1912, xv, 133.
29. Winternitz and Hirschfelder: J. Exper. M., 1913, xvii, 657; 666.
30. Cecil and Blake: J. Exper. M., 1920, xxxi, 403; 1920, xxxii, 1.
31. Roemer: Arch. f. Ophth., 1902, liv, 99.
———: Deutsche med. Wchnchr., 1908, xxxiv, 2, 1977.
32. Foà: Ztschr. f. Hyg., 1893, xv, 369.
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35. Bordoni-Uffreduzzi: Arch. per le sc. med., 1891, xv, 341.
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36. Germano: Ztschr. f. Hyg., 1897, xxvi, 66.
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38. Avery: J. Exper. M., 1915, xxii, 804.
39. Omstead: J. Immunol., 1917, ii, 425.
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41. Klemperer, G., and Klemperer, F.: Berl. klin. Wchnschr., 1891, xxviii, 869.
42. McFadyen: Brit. M. J., 1906, ii, 776.
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### Diplococcus mucosus (Schottmüller)

Encapsulated organisms growing in chains were first isolated in horses by Schütz[1] and by Poels and Nolen[2] and in guinea-pigs by Binaghi[3] who named them *Streptococcus capsulatus*. They were apparently first observed in man

Fig. 47.—Pneumococcus Type III, from twenty-four-hour culture on agar, stained by Huntoon.

Fig. 48.—Pneumoccocus Type III, from twenty-four-hour broth culture, stained by Huntoon.

by Bonome[4] who cultivated them from the exudate in cerebrospinal meningitis. They were subsequently found by Howard and Perkins[5] in a tubo-ovarial abscess. Especial interest was first centered on them by Schottmüller[6]

who found them in a variety of pathological processes in man, peritonitis, meningitis, croupous pneumonia, and named them *Streptococcus mucosus*. The recognition of the organism as a pneumococcus and its importance in producing pneumonia we owe to Longcope.[7] At the present time it is usually classed as a Type III pneumococcus, especially in America.

**Morphology.**—Diplococci and short chains of 6 to 10 individuals surrounded by an easily demonstrable capsule of viscid material both in the tissues and in artificial cultures. The individual cells vary greatly in size, some being twice as large as others. They may measure 1.5 $\mu$ in diameter (v. Lingelsheim). They are usually round, but may be oval like pneumococci. They always appear in pairs. According to v. Lingelsheim the capsule itself is surrounded by a mass of viscid material which shades off into the surrounding medium. The substance of the capsule consists of nucleo-protein, not mucin (v. Lingelsheim).

*Staining Reactions.*—Stained by ordinary anilin dyes but good preparations showing the organisms are sometimes difficult to get because of the abundance of capsular material. If the films are washed in water and then stained the organisms show well. The capsules may be demonstrated by a variety of methods. They take the counterstain frequently in Gram's stain. They are easily demonstrated by the methods of Hiss, Buerger, Hamm, Huntoon, etc.

**Cultural Reactions.**—The organism grows readily on the ordinary laboratory media, its growths being characterized by a viscidity, often well-marked. It grows best at 37° C., slowly at room temperature.

*Agar Plates.*—Colonies delicate, colorless, transparent, glistening, and viscid, about pinhead in size after twenty-four hours at 37° C. Under low power colorless and homogeneous.

*Agar Slant.*—Flat, colorless, transparent expansion, viscid, and sticky.

*Coagulated Hydrocele Fluid.*—Thick, viscid, sticky growth often several mm. thick.

*Hydrocele Fluid Agar.*—Abundant, thick, viscid growth.

*Blood Agar.*—Viscid greenish-grey expansion.

*Serum Agar.*—Growth good like that on plain agar.

*Gelatin Colonies* after three days very delicate, bluish, and glistening.

*Gelatin Stab.*—Small spherical colonies along line of inoculation, after several days yellow-brown. No liquefaction.

Little growth on *potato* or in plain *broth.* Good growth in broth to which blood is added.

*Milk.*—Acid reaction and coagulation. Litmus whey reddened from acid production.

*Hemolysis* usually very slight, but may occur after several days (v. Lingelsheim).

*Pathogenic* to mice and guinea-pigs, especially from substaneous inoculation. Mice develop a fatal septicemia in twenty-four to forty-eight hours after subcutaneous injection of a fraction of an oesa from a serum agar culture. There is a fibrinous or fibrinopurulent exudate at the site of inoculation, in chronic cases a purulent peritonitis and pleurisy.

In man it is found frequently in the upper air passages from which it spreads and causes various types of inflammation such as sinusitis, otitis media, and meningitis, inflammation of the parotid gland, etc. It is of special importance as the cause of a croupous pneumonia which is marked by certain characteristics and is practically always fatal.

1. Schütz: Arch. f. wissensch. u. prakt. Tierh., 1887, 27. 1888, 456.
2. Poels and Nolen: Arch. f. wissensch. u. prakt. Tierh., 1887, 283.
3. Binaghi: Centralbl. f. Bakteriol. [etc.], 1897, xxii, 273.
4. Bonome: Centralbl. f. Bakteriol. [etc.], 1890, viii, 377.
5. Howard and Perkins: J. Med. Research, 1901–02, vi (n. s. i), 163.
6. Schottmüller: München. med. Wchnschr., 1903, l, i, 849; 909.
7. Longcope: Univ. Penn. M. Bull., 1920–03, xv, 51.

### DIPLOCOCCUS LANCEOLATUS OVUM Gärtner

Isolated by Gärtner[1] from septicemia in sheep in which various symptoms occur, endometritis, peritonitis, catarrh of the air passages and enteritis with acute death or death in seven to fourteen days. Probably the same as *Streptococcus ovis* of Wiemann.[2] Organisms found in all the organs in various situations and in the blood. Easily demonstrated morphologically and culturally. They are very abundant in the tissue, appearing as diplococci, short chains, and clumps. According to Gärtner they are lance-shaped and encapsulated. Gram-positive.

Cultural reactions are like those of the streptococci especially in fluid media. Strongly hemolytic. Pathogenic to mice, rabbits, guinea-pigs, and pigeons. Dies out rapidly on

artificial media. Strains vary greatly in virulence under natural conditions which may account for the difference in the character of the lesions.

Gärtner's organism was encapsulated and non-hemolytic, resembling the pneumococcus. Wiemann's organism was powerfully hemolytic and resembled *Streptococcus pyogenes*.

1. Gärtner: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1910, liv, Orig., 546.
2. Wiemann: Ztschr. f. Infektionskr. d. . . . Haustiere, 1911, ix, 233.

### DIPLOCOCCUS GRISEUS NON-LIQUEFACIENS Tissier and Martelly

Described first by Tissier and Martelly[1] in 1902 from putrified meat. Subsequently found by Tissier[2] in war wounds.

**Morphology.**—Large diplococcus occurring as isolated individuals or in short chains. Often in short bacillary forms, probably involution forms.

*Staining Reactions.*—Gram-positive except for the bacillary forms which are Gram-negative.

**Cultural Characters.**—Growth at 22° or 37° C. Agar colonies small, whitish, or bluish-gray, round, regular, later becoming granular. No liquefaction of gelatin. Acid, no gas in glucose. Turbidity and sediment in broth. No coagulation of milk. Turbidity in urine. No acid from lactose. From proteoses in alkaline neutral or faintly acid solution it produces indol, ammonium carbonate, and ammonia. It converts urea into ammonia.

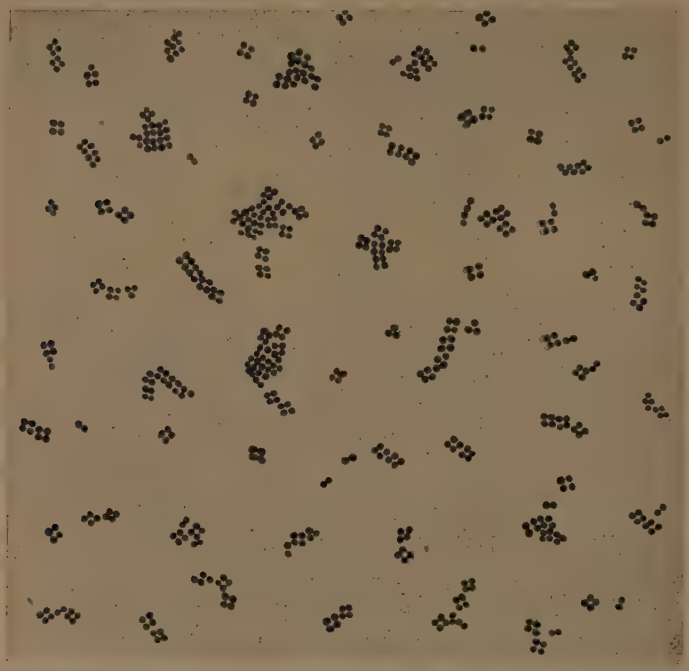

Fig. 49.—*Diplococcus tetragenus* from twenty-four-hour culture on plain agar, stained by Gram.

**Resistance.**—Destroyed by boiling. Ordinary cultures retain their vitality three months.

1. Tissier and Martelly: Ann. de l'Inst. Pasteur, 1902, xvi, 865.
2. Tissier: Ann. de l'Inst. Pasteur, 1917, xxxi, 161.

### DIPLOCOCCUS TETRAGENUS (Gaffky)

Originally found by Gaffky[1] in human sputum. Common in the sputum of tuberculous individuals and in the walls of caseous cavities at autopsy. Spengler[2] believes that this organism may take part in the necrotizing changes

in the living tissue. Frequently found in abscesses (Park), especially those arising in the neighborhood of the respiratory passages. Occasionally found in empyema after pneumonia (Netter). Described by Migula[3] as *Sarcina tetragena* (Gaffky).

Present description based upon the literature and upon the reactions given by a culture from the American Museum.

**Morphology.**—Somewhat large spherical organisms measuring 0.6 to 0.8 $\mu$. According to Gaffky they may be 1 $\mu$ in diameter. The cells are arranged in tetrads surrounded by a mass of viscid material which may be stained with eosin. In cultures the organisms often appear as diplococci, the characteristic tetrads being found chiefly in the tissues. Lehmann and Neumann[4] found typical sarcina packets in old hay infusion cultures. Migula has found similar packets in the tissues. The viscid capsular material is rare in cultures. No increase in size on dextrose agar.

**Staining Reactions.**—Stained easily by the usual anilin dyes. Gram-positive.

*Cultivated* without difficulty on the usual media, best at 37° C.

*Plain Agar Plates.*—Small surface colonies in twenty-four to forty-eight hours, about $\frac{1}{2}$ mm. in diameter. Under low power they are dark, opaque, granular with entire edges. Deep colonies pin-point, under low power, ovals and disks. After three to four days the surface colonies are somewhat larger and may measure 1 mm. in diameter. Older colonies are slightly larger, whitish, and somewhat opaque. Deep colonies when older are distinctly oval, $\frac{1}{2}$ mm. in diameter and tend to break through to the surface.

*Plain Agar Slant.*—Scanty, dull white, non-spreading growth in twenty-four hours, moderately increased in forty-eight hours. In three to four days the growth is denser and somewhat opaque. After eight to ten days a fairly abundant dull-white growth is produced.

*Plain Agar Stab.*—Slight development along the line of inoculation and on the surface in twenty-four hours. In three to four days the line growth is quite dense with a dull-white surface expansion. In old cultures, ten to twelve days, an abundant dull-white surface expansion appears with a fairly abundant line growth.

*Dextrose Agar Plates.*—Colonies develop slowly, appearing in three to four days as round, regular, uniform masses about $\frac{1}{2}$ to 1 mm. in diameter. Under low power they are dark, opaque, granular, with entire edges. Deep colonies pin-point, under low power biconvex disks. After five to six days the surface colonies are somewhat larger, white, and elevated. In old cultures, ten to twelve days, the surface colonies are 1 to 2 mm. in diameter, white, elevated, frequently umbilicated. Deep colonies oval, tend to break through to the surface and may be $\frac{1}{2}$ mm. in size. Agar moderately acid.

*Dextrose Agar Slant.*—Scanty dull white growth in twenty-four hours, somewhat increased in forty-eight hours. In three to four days the growth is thicker, china-white in color. The agar becomes highly acid. In old cultures the growth is thick, white, and abundant.

*Gelatin Plates.*—Surface colonies grow slowly, appearing on the third and fourth day. They are whitish, glistening, slightly raised masses about $\frac{1}{2}$ mm. in diameter. Under low power they are round, regular, granular, with entire edges. Older gelatin colonies may be 1 to 2 mm. in size and show slightly fringed edges. Deep colonies when young are small, whitish points, under low power round with entire edges. When older, dark brown in color and somewhat irregular in contour. No liquefaction.

*Gelatin Stab.*—Delicate line growth in two to three days with a whitish, viscid, glistening expansion on the surface. No liquefaction.

*Blood-serum.*—Faint, delicate, whitish growth in three to four days, not increasing in extent.

*Potato.*—Usually a faint whitish expansion. In some strains an abundant, thick, white, very viscid mass, from which long threads may be drawn off with the platinum needle (Migula).

*Litmus Milk.*—Usually no change.

*Broth.*—Faint turbidity and sediment in two to three days. No scum. In old cultures the sediment may be marked and the broth clear.

*Peptone.*—Faint turbidity and sediment. No scum. Sediment increased in old cultures with a clearing of the solution.

*Smith Fermentation Tubes*:

*Dextrose.*—Turbidity and sediment in bowl, arm clear. No scum. The growth tends to remain restricted to the bowl although the reaction becomes highly acid.

*Saccharose.*—Turbidity and sediment. No scum. Arm clear. Reaction acid.

*Lactose.*—Turbidity and sediment. No scum. Arm clear. Reaction acid. No production of indol. No utilization of ammonium salts as the only source of nitrogen (Hucker).

**Pathogenic Action.**—Highly virulent to white mice. After inoculation of small quantities the mice develop inactivity and somnolence in two to three days and practically always die in four to six days. At autopsy the organisms are found chiefly inside the smaller blood-vessels,

especially in the spleen, kidneys, liver, and lungs. They are rare in the heart's blood. Gray mice are practically always immune to this organism. Guinea-pigs develop local abscesses or septicemia from subcutaneous injection. After intraperitoneal inoculation a purulent peritonitis develops with large numbers of encapsulated organisms in the exudate. Rabbits and dogs immune.

Certain strains of *Diplococcus tetragenus* appear to have no pathogenic action. It is doubtful whether these represent the original organism of Gaffky.

1. Gaffky: Arch. f. klin. Chir., 1883, xxviii, 500.
2. Spengler: Ztschr. f. Hyg., 1894, xviii, 343.
3. Migula: p. 225.
4. Lehmann and Neumann: Atlas and Principles of Bacteriology. 2. Aufl. Weaver Translation, 1901, ii, 171.

### Micrococcus ovalis Escherich

Found by Escherich in the meconium, in stools of children fed on milk, and in the intestinal canal of sucklings. Occasionally found in the normal stools of children and adults. Usually regarded as the same as the "enterococce" of the French writers. Present description from a personal isolation.

Fig. 50.—*Micrococcus ovalis*, isolated from intestinal contents, twenty-four-hour culture on agar, stained by Gram.

**Morphology.**—Small cocci varying greatly in size in different strains. According to Escherich they are 0.2 to 0.3 $\mu$ in diameter. In many instances they are larger, measuring 0.5 to 0.6 $\mu$ in diameter. Oval forms are common, measuring 0.3 $\mu$ in breadth and 0.6 to 0.7 $\mu$ in length (Escherich). The organisms are frequently arranged as pairs, the elements of which are joined at an acute angle. These are described as "saddle-back" forms. Short chains and small bunches are not uncommon. Slightly enlarged on dextrose agar.

**Staining Reactions.**—Stains easily by ordinary anilin dyes. Gram-positive.

*Cultivated* easily on ordinary media. Growth best at 37° C.

*Plain Agar Plates.*—Colonies appear in twenty-four hours as small pin-points, and are better developed in forty-eight hours when they may be $\frac{1}{2}$ mm. in diameter. Under low power they are pale, granular, nucleated, with thin entire edges. Deep colonies pin-point, under low power ovals and disks. In four to five days the colonies are somewhat larger, often 1 mm. in diameter. After ten to twelve days the maximum growth is attained. The colonies are now 2 to $2\frac{1}{2}$ mm. in size, dull-white and opaque.

*Plain Agar Slant.*—Delicate, whitish, non-spreading growth in twenty-four hours, much like that of the streptococci. In three to four days the growth is somewhat heavier, but is still delicate and does not spread. After eight to ten days it is much the same.

*Plain Agar Stab.*—Very delicate line and surface growth in twenty-four hours. The growth increases slightly in three to four days and becomes more abundant after ten to twelve days.

*Dextrose Agar Plates.*—Small, delicate, whitish surface colonies in twenty-four hours about ½ mm. in diameter. Under low power they are somewhat opaque, denser in the center, with thin entire margins, and may be nucleated. Deep colonies pin-point, under low power ovals and disks. Bottom colonies often thin and tend to spread, with irregular margins. Considerable acid develops slowly and in three to four days all the plates have an acid reaction. Colonies three to four days old are ½ mm. in size, dull-white and opaque. Older surface colonies may be definitely nucleated. Deep colonies when five and six days old are distinctly oval or disk-shaped to the unaided eye.

*Dextrose Agar Slant.*—Delicate, whitish, non-spreading growth in twenty-four hours with a beginning acid reaction. In three to four days the growth is thicker and the agar highly acid. After ten to twelve days the growth is much the same.

*Gelatin Plates.*—Colonies develop very slowly and are usually visible in four to five days. They are small and fine, under low power round, regular, granular, with entire margins and a slightly yellowish color. After seven to eight days they may attain a diameter of ½ mm. No liquefaction.

*Gelatin Stab.*—Faint line growth, barely visible in twenty-four hours, no surface growth. After five to six days the line growth is thicker and there is a slight surface expansion. No liquefaction. According to Escherich the growth in the depths of gelatin often resembles small white buttons.

*Blood-serum.*—Faint, delicate, non-spreading growth, not characteristic.

*Potato.*—Usually no growth. Escherich reported a somewhat luxuriant growth in his cultures on potato, made up of small delicate colonies.

*Litmus Milk.*—Very slow development of an acid reaction and a whitish sediment. The milk seldom coagulates spontaneously, but in old cultures may coagulate on heating.

*Broth.*—Faint turbidity and sediment after two to three days. No scum. The turbidity and sediment increase slowly and may be marked in old cultures. The broth does not clear.

*Peptone.*—Turbidity and sediment. No scum. Peptone solution does not clear.

*Smith Fermentation Tubes:*

*Dextrose.*—Faint turbidity in bowl and arm in twenty-four to forty-eight hours, with a granular sediment. The turbidity increases slowly and the sediment tends to run up into the arm. No scum. Reaction acid.

*Saccharose.*—Faint turbidity in bowl and a granular sediment. Arm usually clear. No scum. No acid.

*Lactose.*—Growth more abundant than in dextrose on saccharose. Dense turbidity in bowl and arm. Granular sediment running up into arm. No scum. Reaction acid. Escherich reports a luxuriant growth in lactose solutions with a white, cloudy precipitate.

Non-pathogenic.

Escherich: Die Darmbakterien des Säuglings. 1886, p. 89.

### DIPLOCOCCUS GRISEUS LIQUEFACIENS Tissier

First isolated by Tissier from a breast-fed and from a bottle-fed infant, both suffering from severe gastro-intestinal disturbances.

**Morphology.**—Cocci varying in size from 0.5 to 1 $\mu$ in broth cultures twenty to twenty-four hours old, arranged as single elements or in pairs with the proximal surfaces flattened. Also in short chains of 4 to 6 elements. In agar, gelatin, or potato cultures the diplococcus form predominates.

**Staining Reactions.**—Gram-positive.

*Destroyed* in fifteen minutes by heating to 60° C.

*Survives* in gelatin tubes two months or more.

**Cultural Reactions.**—Grows well under aërobic and anaërobic conditions at 20° to 22° C., but better at 37° C.

*Plain Agar.*—Growth slow. At the end of twenty-four hours at 37° C. it appears as small, gray, pin-point colonies with turbidity and powdery sediment in the water of condensation. In forty-eight hours colonies vary in size from 1 to 1½ mm. They are grayish, transparent, moist, slightly raised, with fine entire edges.

*Broth.*—Delicate, uniform, whitish turbidity in twenty-four hours. In three days broth cleared with a granular deposit at the bottom of the tube.

*Gelatin Plates.*—Colonies appear in forty-eight hours like those in agar. Gelatin slowly liquefied.

*Gelatin Stab.*—Delicate line growth with a funnel-shaped liquefaction at the bottom of which a round, grayish-white mass collects. Liquefaction complete in eight to ten days.

*Milk.*—Acidity and coagulation in four days without retraction of the clot. At the end of eight days the clot expresses a clear transparent colorless liquid.

*Potato.*—Fine, moist, whitish growth in three days without blackening of the potato.

*Deep Sugar Agar.*—Fine, small, grayish-white colonies distributed regularly in the lower part of the agar, but more abundant in the acid zone.

**Not pathogenic** for mice.

**Differentiated** from the *Streptococcus coli gracilis* which forms thicker colonies on agar, coagulates milk only after a long time and forms long chains of 6 to 20 elements 2 to 4 $\mu$ in diameter, S-shaped. Differentiated from *Streptococcus coli brevis* which forms olive-green colonies on gelatin and lemon-yellow colonies on agar.

Tissier: Recherches sur la flore intestinale des nourrissons. Paris, 1900, p. 46.

### DIPLOCOCCUS FŒTIDUS (Klamman)

Originally obtained from the posterior nares in man by Klamman and called by him *Diplococcus fluorescens fœtidus.*

**Morphology.**—Spherical organisms in single elements, pairs, short chains, and irregular groups. The diplococci measure 1.4 $\mu$ in diameter. Chains of 8 to 10 elements in gelatin cultures.

**Cultural Characters.**—Grows rapidly on the usual media, best at 37° C. also at 12° to 15° C.

*Agar Slant.*—Whitish irregular layer.

*Gelatin Plates.*—Superficial colonies gray or brown with smooth edges liquefying the gelatin in crater-like depressions. Brownish-gray sediment. Adjacent gelatin grass-green to violet in color.

*Gelatin Stab.*—Roundish, flat, pale gray, mussel-like depression with cylindrical liquefaction. On the surface an opalescent iridescent scum with a grayish-green sediment. Complete liquefaction, the upper part of the gelatin green with a violet membrane.

*Potato.*—Brownish-green finely granular growth turning dark blue-green. The adjacent potato turns blue.

*Blood-serum.*—Gray-green growth with liquefaction.

Produces a sweetish, peculiarly aromatic, strongly ammoniacal odor in all cultures.

Klamman: Allg. med. Centr-Ztg., 1887, p. 1347.

## GENUS 3. LEUCONOSTOC Van Tieghem (emended S. A. B.)

Saprophytes, usually growing in cane-sugar solutions. Cells in chains or pairs, united in large zoöglœal masses. Some types at least Gram-negative.

Type species *Leuconostoc mesenteroides* (Cienkowski), Van Tieghem.
" Aller (Zettnow), Bergey.
" Opalanitza (Zettnow), Bergey.

### LEUCONOSTOC MESENTEROIDES (Cienkowski) Van Tieghem

Cultivated by Van Tieghem[1] from the white gelatinous masses found in beet-sugar solutions in the process of manufacture, known in Germany as "Froschlaiche" and in France as "gomme de sucrerie." They were first observed by Scheibler,[2] by Jubert,[3] and by Mendès.[4] Their bacterial nature was definitely established by Cienkowski,[5] who named the organism causing them *Ascoccus mesenteroides*, and by Van Tieghem who named it *Leuconostoc mesenteroides*, because of its white color and its resemblance to the form described as "Nostoc."

**Morphology.**—Spherical forms, measuring 1.8 to 2 $\mu$, enclosed in homogeneous protoplasm which forms a thick, tough gelatinous capsule. These spherical forms divide into twos, fours, and eights. Later they form a kind of compact, mammillated, worm-like tubercle. These tubercles increase in size, unite and form the gelatinous masses known as "gomme de sucrerie." They are polyhedral in form and give a kind of parenchymatous structure to the

mass. The spherical forms are smaller than *Micrococcus ureæ*, and in addition elongated bacillary forms may be found. The gelatinous capsule is six to ten times the diameter of the spherical forms, measuring 6 to 20 $\mu$. It is elastic, almost cartilaginous, highly refractile, viscid, insoluble in water, perfectly white. Its chief chemical ingredient is $C_{12}H_{10}O_{10}$, called dextrane by Scheibler. The cells themselves contain a nitrogenous substance called "bétaïne" by Van Tieghem. In old material which has ceased to multiply a kind of spore appears in the chains of organisms inside the capsule, thick and tough, from which the spherical forms are generated. This species contains no chlorophyl.

Cultivated artificially in a variety of beet-sugar solutions. Kept with free access of oxygen, inverting the saccharose to glucose and levulose. Bergey[6] states that this species grows in dextrose gelatin as small white to grayish-white, raised nodular colonies, and along the entire dextrose gelatin stab without liquefaction.

1. Van Tieghem: Ann. d. sc. nat. S. 6, Bot. 1878, vii, 180.
2. Scheibler: Vereinszeitschrift für die Rübenzuckerindustrie, Bd. 24, 1874.
3. Jubert: J. d. fabric. de sucre, December 31, 1874.
4. Mendès: J. d. fabric. de sucre, April 22, 1875.
5. Cienkowski: Ueber die Gallertbildungen des Zuckerübensaftes. Kharkow. 1878.
6. Bergey: Determinative Bacteriology. 1923, p. 45.

#### LEUCONOSTOC ALLER (Zettnow) Bergey

Described by Zettnow in saccharose solutions containing "Froschlaiche" as *Streptococcus Aller* and isolated on saccharose gelatin plates.

Zettnow: Ztschr. f. Hyg., 1907, lvii, 154.

#### LEUCONOSTOC OPALANITZA (Zettnow) Bergey

Described also by Zettnow. Differs in a few cultural features from *Leuconostoc Aller*.

Zettnow: Ztschr. f. Hyg., 1907, lvii, 154.

### GENUS 4. STREPTOCOCCUS Rosenbach, emended Winslow and Rogers

Chiefly parasites. Cells normally in short or long chains (under unfavorable conditions, sometimes in pairs and small groups, never in large packets). *Generally stain by Gram.* Capsules rarely present, no zoöglœal masses. On agar streak, effused translucent growth, often with isolated colonies. In stab culture, little surface growth. Many sugars fermented with formation of large amount of acid, but inulin is rarely attacked. Generally fail to liquefy gelatin or reduce nitrates.

1. *Non-liquefying*

Type species *Streptococcus pyogenes* Rosenbach.
" *hemolyticus* Schottmüller.
" *viridans* Schottmüller.
" *erysipelatos* Fehleisen.
" *epidemicus* Davis and Rosenow.
" *pyogenes bovis* Glage.
" *equi* Schütz.
" *lacticus*.

2. *Liquefying*

*Streptococcus coli gracilis* Escherich.
" *coli brevis* Escherich.
" *enteritidis* Escherich.
" *liquefaciens* Sternberg.
" *septicus* (Babes) Migula.

*Gram-negative Streptococci*

*Streptococcus mastitidis* (Guillabeau) Migula.
" of Doléris and Bourges.
" of Etienne.
" of Cottet and Tissier.
" of vaginal catarrh of cattle von Ostertag.
" of infectious abortion of mares von Ostertag.

**The Streptococci.**—Streptococci were originally observed in association with staphylococci by Rindfleisch, v. Recklinghausen, and many others in focal necroses in the organs of individuals dead of sepsis or pyemia, and probably first regarded as a specific micro-organism by Klebs. The distinct characteristic of growing in chains was first clearly recognized by the surgeon Billroth, to whom we owe the designation "chained coccus." The differentiation between staphylococcus and streptococcus was established by Ogston, who associated these two types of micro-organisms with two types of pathological processes, the localized phlegmon and the spreading infection like erysipelas. Streptococci were first obtained in pure cultures by modern bacteriological methods by Fehleisen[1] from erysipelas, subsequently by Rosenbach[2] from wounds. The *Streptococcus erysipelatos* of Fehleisen was later proven to be identical with the *Streptococcus pyogenes* of Rosenbach on the basis of cultural reactions and animal pathogenicity. Since that time the term *Streptococcus pyogenes* has come into general use to designate that type of Gram-positive coccus growing in chains responsible for a great variety of lesions in man and animals characterized by their tendency to spread and result in septicemia.

### STREPTOCOCCUS PYOGENES Rosenbach

**Morphology.**—Small cocci, varying in diameter from 0.6 to 1.5 $\mu$ and growing in chains both in the animal body and on artificial media. The length of the chains varies greatly. It may consist of 3 to 4 elements or of several hundred and shows all grades between the short and the long chains. In cultures the length of the chain varies with the media. Films taken from the surface of solid media frequently show bunches of spherical elements arranged much like staphylococci, with only here and there a few short chains made up of 3 to 4 to 10 to 12 elements. In fluid media, broth or the water of condensation from agar, long chains predominate, often made up of several hundred individuals. The care with which films are made determines to some extent the length of chains found on examination. If the films are rubbed up a good deal to facilitate their drying, the chains are frequently broken and only bunches of spheres are found with here and there a few short chains. If the material is allowed to flow gently over the coverslip or slide and dried without touching the number of chains left uninjured is much greater. Even when the greatest care is taken to avoid manipulation some strains show a marked tendency to grow in long chains, while others grow in short chains of only 5 to 6 elements. This difference in chain formation has not thus far been definitely associated with any other characteristic, such as virulence, and is of little or no value in determining the source of the cultures. In many instances the organisms appear as distinct tangles of single cells and long and short chains.

V. Lingelsheim[3] states that the morphology of the streptococcus is best seen in neutral or slightly alkaline 1 per cent. peptone broth containing 5 to 7.5 c.c. normal sodium hydrate per liter. A higher percentage of alkali, 10 to 15 c.c. normal sodium hydrate, to neutralize any acidity formed, is also valuable for certain strains.

The individual cells are usually about 0.5 to 0.6 $\mu$ in diameter. They may be much larger, up to 1.5 $\mu$ in size, but such large forms are exceptional. In glucose broth and glucose agar they are often a trifle larger and also in slightly acid broth. They vary greatly in size in fluid media and in broth very minute spherical forms, much smaller than the ordinary cell, can sometimes be made out.

Under many circumstances streptococci lose their typical morphology and take on aberrant shapes. The individual elements are often somewhat elongated, so much so indeed as to look almost like very short bacilli. Such elongated forms are possibly more common in saprophytic species but they occur in true pathogenic varieties. They are more common in old cultures, in cultures long under artificial cultivation and in fluid media. In other instances the cells are swollen and distorted and lose their resemblance to spheres. Such involution forms

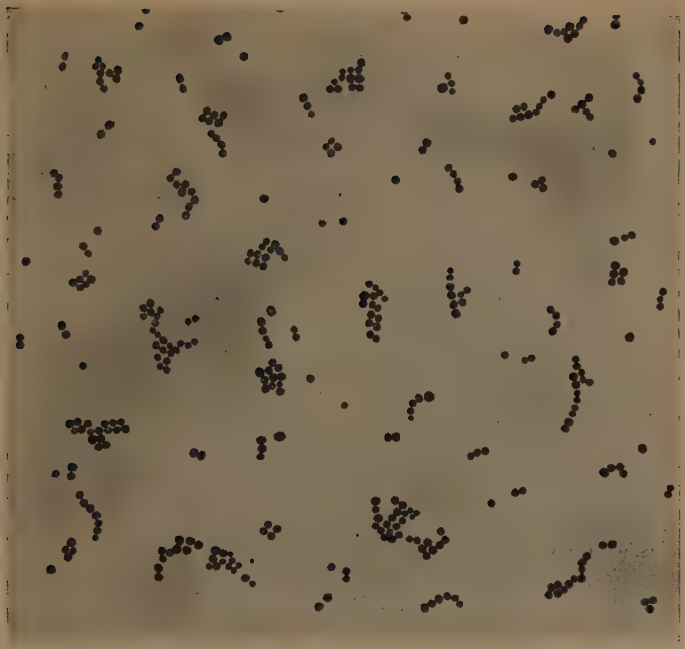

Fig. 51.—*Streptococcus pyogenes* from forty-eight-hour culture on plain agar, stained by Gram.

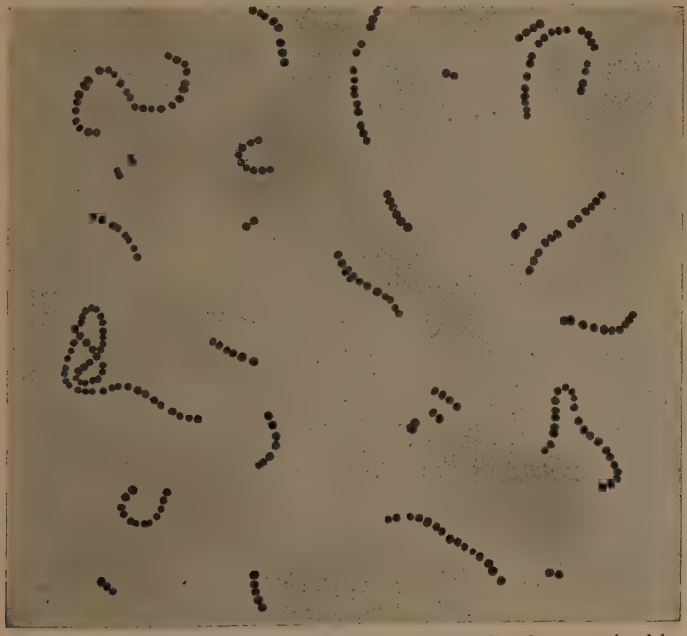

Fig. 52.—*Streptococcus pyogenes* from forty-eight-hour broth culture, stained by Gram.

are common in cultures but somewhat rare in streptococcus lesions. In old abscesses, however, and in the purulent hemorrhagic secretions in streptococcus pneumonia large distended organisms can often be seen which bear the closest resemblance to the involution forms seen in cultures.

Non-motile. No spores. No capsules.

**Staining Reactions.**—Stains readily with all the anilin dyes, especially well with methylene-blue (when good dyes are available), gentian-violet, safranin or dilute carbolfuchsin. Always strongly Gram-positive. Easily demonstrated in pus or exudates by making ordinary films. For demonstration in hardened tissues Gram's or Weigert's methods give the best results although a great variety of other procedures have been suggested.

**Cultivation.**—The streptococci grow delicately upon artificial media and considerable care must be exercised in obtaining them and in keeping them alive. Some strains grow readily from the first and can be transferred almost indefinitely. Others are accustomed to

Fig. 53.—*Streptococcus pyogenes* from a throat culture showing long chains.

artificial conditions only with difficulty but after once obtained may be kept alive easily. Some streptococci, however, are very rigid in their growth requirements and artificial cultures often fail completely. In all cases growth is scanty compared with that of the ordinary pathogens and resembles markedly the growth of the pneumococcus and gonococcus.

The *Streptococcus pyogenes* grows best at 37° C. and at temperatures near this. It grows slowly at room temperature, little or not at all at temperatures below 20° C. and above 40° C. It grows best as an aërobe, but the oxygen requirements of different strains vary and some strains develop better under partial tension. By the addition of reducing substances like sodium sulphate or neutral red to media anaërobic growth is favored. Cultures are best kept alive under anaërobic conditions at low temperatures as that of the ice-chest. The organisms

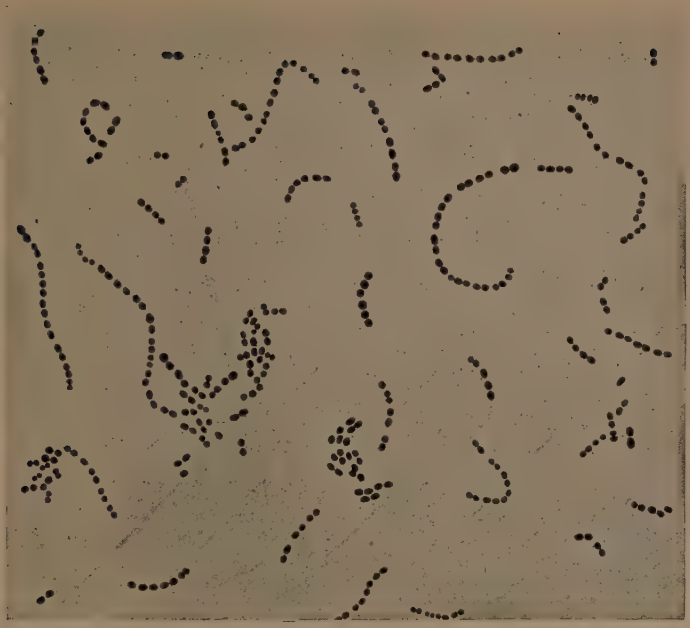

Fig. 54.—*Streptococcus viridans*, seven-day culture on blood agar, isolated from the mouth, stained by Gram.

Fig. 55.—*Streptococcus hemolyticus* from sore throat, blood agar culture twenty hours old, stained with methylene-blue. Mixed with bacilli.

usually survive five to six weeks on agar and in broth at room temperature. Rarely a few organisms may be alive after longer periods, sometimes three months. Mixtures of serum and broth are well adapted for this. In general cultures should be transferred at frequent intervals, ten days to two weeks.

Dried on pieces of cloth they may survive some time five to six weeks (Kurth). Sprayed on fine particles of dust they survive in diffuse daylight ten days (Kirstein[4]). They have been reported as somewhat resistant by Germano[5] when culture material or pus is dried. When blood or pus is present the organisms survive longer in the dark than in the light and seem to be destroyed more certainly by rapid drying than by slow drying. They are readily destroyed by the ordinary disinfectants, by HCl 1 to 250, $H_2SO_4$ 1 to 250, sublimate 1 to 2500, copper sulphate 1 to 200, carbolic acid or cresol 1 to 250, lysol 1 to 300. Killed at 65° C, in two hours at 70° C. in one hour (v. Lingelsheim).

**Cultural Characters.**—*Agar colonies* small, isolated, grayish or grayish-yellow points which seldom are more than 0.5 mm. in diameter in forty-eight hours. Under low power the superficial colonies are round or almost round, finely granular, occasionally with radial markings. Edges wavy. Deep colonies pin-point, under low power brownish, round, or whetstone-shaped, without detail. Old colonies may be 1 mm. in diameter, sometimes dry and granular.

*Agar Slant.*—With moist agar and an abundance of material for transfer the growth appears in two to three days as fine, grayish-white, opaque, more or less confluent colonies frequently forming a gray transparent expansion. Growth always more abundant in water of condensation where it forms a turbidity and on the agar near this fluid.

*Agar Stab.*—Growth as spherical grayish-yellow isolated colonies or as a confluent streak. The growth may be more abundant in the upper part of the stab and about the point of puncture or in the deeper part, depending upon the oxygen requirements of the particular strain.

Various substances may be added to the agar to enhance the growth of the streptococcus. The best of these are ascitic fluid, hydrocele fluid, human blood-serum, serum from animals, whole defibrinated blood of man or animals.

*Broth.*—Growth as a slight turbidity in twenty-four to forty-eight hours with a delicate sediment. The turbidity increases and the sediment becomes more marked. With greater peptone content, up to 3 to 5 per cent., and with an infusion made from autoclaved meat at 150° C. the growth is much more abundant (v. Lingelsheim). Scum formation practically always lacking.

*Sugar Broth.*—Growth more abundant in dextrose broth (0.1 to 2 per cent.). The acidity produced from the sugar must be neutralized otherwise the organisms die. This may best be done by the addition of calcium carbonate in the form of marble dust as first proposed by Hiss.[6] The sediment is often marked in sugar broth due to the coagulation of the protein from the acidity.

*Serum-broth.*—The addition of human blood-serum, rabbit serum, horse serum, defibrinated blood or ascites fluid favors the development of streptococci. Human serum may be added in the proportion of 2 to 3 parts to one part of broth, horse serum in proportion of 2 parts to one of broth. Growth also takes place in undiluted rabbit serum. In fluid serum media the formation of the sediment is more marked.

*Peptone Solution.*—Delicate growth, slight turbidity and faint sediment in 1 per cent. solution. No scum.

*Coagulated Blood-serum or Loeffler's Serum.*—Growth often fairly luxuriant as small isolated colonies or as a delicate expansion difficult to see in the first few days. Growth sometimes abundant in the water of condensation of freshly prepared serum.

*Glucose Litmus Agar Plates.*—Colonies appear in twenty-four to forty-eight hours, small and delicate, much like plain agar colonies, but more abundant. They measure about ½ to 1 mm. in twenty-four to forty-eight hours. Under low power they are round, regular, granular with entire edges. Deep colonies small, fine, pin-point, under low power round or oval, granular, opaque. About each colony the agar becomes acid and often the whole plate is acid in forty-eight to seventy-two hours. The acidity is permanent.

*Glucose Litmus Agar Stab.*—Moderate growth along line of inoculation with scanty surface growth in twenty-four to forty-eight hours. The agar is turned acid, the acidity being permanent.

*Glucose Agar Stab.*—Abundant growth with the production of acid. This acid produces a turbidity and converts the agar to a whitish opaque mass (v. Lingelsheim). This is due to the action of the acid upon the agar colloids.

*Gelatin colonies* are like those on agar, small, spherical, often transparent, never more than 0.5 mm. in size. They reach their maximum of growth in two to three days. No liquefaction.

*Gelatin Stab.*—Faint delicate growth along line of inoculation with occasionally a slight surface expansion. Like the growth in agar stabs, the part of the gelatin in which the growth is most abundant depends on the oxygen requirements of the particular strain.

*Potato.*—Growth usually very delicate or entirely lacking. It is sometimes manifest as a faint grayish expansion, appearing only after several days' cultivation at 37° C.

*Milk.*—Growth usually abundant with a faint acidity slowly leading to coagulation. Some strains fail to grow or grow so slightly as never to reveal an acid reaction.

*Dextrose Fermentation Tubes.*—Slight turbidity in bulb slowly extending up into the closed arm. Sometimes a delicate sediment just at the bend of the tube. Moderate amount of acid always produced.

This acid is lactic acid, both the active and inactive varieties. Various indicators such as neutral red, rosolic acid, etc., may be added to the broth to indicate the acidity. Acidity also in lactose, sometimes in saccharose.

Langwill[7] has found considerable difference in acid production by different types of streptococci. Thus in dextrose, saccharose, and lactose broth the acid production by non-hemolytic streptococci was found to be twice as great as that of hemolytic streptococci in the first twenty-four hours. At the end of the period of acid production, however, the titratable acidity and hydrogen-ion content were about equal in the two groups. The hemolytic streptococci require a longer period of time in which to bring about a complete conversion of the sugar to acid. Non-hemolytic streptococci produce lactic, formic, acetic, and a possible trace of butyric acid while hemolytic streptococci and *Streptococcus viridans* produce lactic, acetic, and propionic acid with probably traces of butyric acid, but no formic acid.

*Blood Agar.*—Growth as very fine dry colonies somewhat difficult to see in the first twenty four to forty-eight hours. The blood in the immediate vicinity slowly clears in the majority of strains. Growth is better in the water of condensation which becomes turbid.

**Hemolysins or Hemotoxins.**—The capacity of *Streptococcus pyogenes* to dissolve blood-corpuscles both in the tissues and in "vitro" was first noted by Marmorek.[8] This hemolytic property of the streptococci was emphasized by Schottmüller[9] who employed blood agar (2 c.c. blood to 5 c.c. agar) for this purpose. On plates the colonies are surrounded in eighteen hours at 37° C. by a light broad zone in which the hemoglobin has left the corpuscles and diffused into the surrounding medium. On blood agar streaks the hemolysis is noted by a gradual clearing of the agar in the immediate vicinity of the growth.

Hemolysis also takes place in fluid media (*i. e.*, broth) to which blood is added. Streptococci grown in such media produce a complete laking of the blood in twenty-four hours; at this time the laked blood takes on a brownish-red color due to methemoglobin (Rieke[10] Grüter[11]). Kerner states that the streptococcus hemolysin acts most strongly on dog's blood, mostly weakly on human and frog's blood. This hemolysin is very labile and is destroyed at 55° to 60° C. in fifteen to thirty minutes. It is also destroyed by centrifugation and lost in filtration.

Broth cultures may also be tested by hemolysin by adding various quantities to fixed quantities of defibrinated whole or washed blood. It is not especially powerful, the strongest being one in which about 0.1 c.c. lakes 1 c.c. of a 5 per cent. suspension of washed corpuscles. Many attempts to obtain the hemolysin free from organisms have been made. Owing to the lability of the substance and its absorption by the filters, the filtrates have much less strength than the cultures. Filtrates with a strength such that 0.03 c.c. will lake 1 c.c. of washed rabbit corpuscles may be obtained if great care is taken in manipulation (Robinson and Meader[12]).

**Pathogenic Action.**—*Streptococcus pyogenes* varies greatly in virulence under artificial conditions, some cultures having little or no action on animals. The virulence is also rapidly lost on cultivation, often in 2 to 3 generations. Mice are the most susceptible of laboratory animals. Subcutaneous injection produces a fatal infection in one to four days. At autopsy there is some edema and hemorrhage at the site of inoculation, the adjacent lymphatic glands are enlarged. The gross pathological changes are not marked however. On microscopic examination there is an abundant local growth of the organisms which also abound in the blood of the general circulation and in all the organs. With organisms of less virulence local abscesses develop and the animals survive ten to fourteen days before fatal septicemia develops. When the organisms are of still weaker virulence little or no effect may be noticeable beyond a local abscess which breaks down and heals or an infiltration with loss of hair.

Intraperitoneal inoculation leads at times to a fatal peritonitis and intravenous inoculation to rapidly fatal septicemia.

In rabbits subcutaneous inoculation leads to fatal septicemia with an extensive exudate at site of inoculation in some instances, with almost no local changes in others. There may be a serous or sero-sanguinous exudate in the serous cavities, swelling of spleen not usually marked, injection of vessels of intestines and cloudy swelling of kidneys. Intraperitoneal injection leads to severe peritonitis with sero-sanguinous or purulent exudate. By cutaneous injection, especially in the ear, spreading inflammation develops with pathological changes in the tissues analogous to those in human erysipelas (Fehleisen). With intraperitoneal, intrapleural and intravenous inoculation various organs show areas of inflammation, especially the liver and kidneys, the joints and the endocardium. According to v. Lingelsheim cultures containing clumps of organisms tend to localize in the lungs.

With cultures of low virulence the animals survive some weeks or months, dying eventually from cachexia due to toxemia. They may show diarrhea and muscle atrophy especially in the hind legs. Rabbits may also be infected per os (Tonarelli, Bail[13]), developing an acute enteritis and fatal general infection. Lexer has reported infection of rabbits through the intact tonsil.

Other animals such as guinea-pigs, rats, cats, dogs, horses, cattle, sheep, and goats are almost absolutely refractory to cultures immediately after isolation. They may rarely show a rise of temperature and local abscesses at site of inoculation. Recently Bayne-Jones[14] has isolated a typical hemolytic streptococcus from a cat.

**Virulence.**—According to v. Lingelsheim *Streptococcus pyogenes* (*i. e., longus*) may be regarded as highly virulent when 0.01 c.c. of a well-developed broth culture kills a mouse of 20 grams or a rabbit of 1000 grams weight by subcutaneous inoculation in one to four days, by intraperitoneal injection in doses of 0.5 to 1 c.c. in eight days. From a virulence of this grade down to practically no virulence cultures of every grade of pathogenicity may be encountered. The virulence diminishes rapidly under artificial conditions. It is retained best in defibrinated blood in serum and in serum-broth. By rapidly passing streptococci through susceptible animals the virulence can be greatly augmented. Thus Knorr,[15] Petruschky,[16] Aronsohn[17] and others have obtained cultures of such virulence that $\frac{1}{1000000}$ c.c. of broth culture sufficed to kill mice or rabbits. Such highly virulent strains are also virulent for other animals like guinea-pigs and rats. Occasionally animal passage of certain strains of streptococci of low virulence results in a complete loss of virulence in them.

**Toxins.**—Filtrates from broth cultures of *Streptococcus pyogenes* in large doses have been found toxic to small animals by a number of observers (Roger[18], Marmorek, Baginsky and Sommerfield[19]). Other investigators have employed chemical methods to obtain toxic substances. Thus Laitinen[20] used ammonium sulphate and obtained a poison which killed rabbits in doses of 0.1 to 0.4 grams when injected intraperitoneally and Marmier precipitated cultures with alcohol and obtained a preparation of which 0.01 gram was fatal to rabbits. In other instances toxic materials have been found in cultures of streptococci in leukocytes and immune serum (Marmorek) and in broth to which intraperitoneal exudate due to streptococcus has been added (v. Lingelsheim). Bardeleben found poisons in the exudate produced by the injection of aleuronate followed by streptococci. As v. Lingelsheim points out the toxic substances seem to be produced by the streptococcus only under the influence of the animal tissues. Such toxins as have been found are sometimes destroyed at 60° C. (Roger and Marmorek), sometimes resist this temperature (v. Lingelsheim) and occasionally withstand boiling (Baginsky). In many instances filtrates from virulent streptococci are quite without action on animals (Aronson).

Recently Zinsser[21] has found toxic substances in streptococcus cultures in "hormone" broth with or without defibrinated blood and in young agar cultures. These toxic materials, called x substances, are not regarded by Zinsser as specific streptococcus products, but as representatives of a group of poisonous bodies produced by a number of pathogenic and non-pathogenic bacteria.

**Lesions in Man.**—*Streptococcus pyogenes* is responsible for lesions of the greatest diversity in man, ranging from the mildest type of local inflammation to rapidly fatal septicemia. The organisms from such diversified lesions vary greatly in pathogenicity for animals, but not to the extent that the character of the lesions would suggest. The course and outcome of streptococcus infection evidently depend upon the resistance of the individual to the infection as well as upon the virulence of the particular strain by which he is attacked. In the skin and subcutaneous tissues the most important lesion is erysipelas which is practically always due to this organism. It may vary in severity from simple exudates in the rete Malpighii to deep seated purulent and phlegmonous inflammations. The disease is directly transmissible from individual to individual. The organisms producing erysipelas, however, can produce quite different types of inflammation when transmitted to others, as for instance, puerperal fever. At the same time genuine erysipelas can be produced by streptococci derived from quite different diseases, as scarlet fever (Heubner). Lymphangitis is often due to the streptococcus. From the skin the streptococcus can invade the blood-stream producing a deadly septicemia or a more chronic disease in which various structures are involved, particularly the serous membranes, resulting in pleurisy, pericarditis, (peritonitis), endocarditis, arthritis, etc. Streptococcus infections are particularly common in inflammations of the throat and vary from a mild pharyngitis to a severe angina with an extensive membrane. From these lesions the organisms may invade the blood-stream producing a fatal septicemia or chronic inflammation of the joints, the heart valves, etc. Such types of infection are frequent in milk-borne streptococcus disease.

The streptococcus is the cause of a considerable proportion of cases of puerperal fever, especially the severe type with blood invasion. It is occasionally the cause of appendicitis and peritonitis. Rarely it may invade the conjunctiva, producing a severe conjunctivitis. It is especially active in the production of pneumonia, as a rule in the course of other diseases like influenza, this pneumonia being characterized by distinct pathological changes (MacCallum). Streptococci are also found in many cases of diarrhea and dysentery particularly in young children.

**Classification of Streptococci.**—Numerous attempts have been made to classify the streptococci which occur in diversified lesions and to establish some differential criteria by means of which they can be separated from the large number of non-pathogenic saprophytic strains. No classification has been found entirely satisfactory and for the present we must regard all the varieties which have been described as members of the group *Streptococcus pyogenes*.

V. Lingelsheim divided streptococci according to the length of chains, considering the long-chained forms, *Streptococcus longus*, as the pathogenic varieties and the short-chained forms, *Streptococcus brevis*, as in general non-pathogenic or saprophytic. As pointed out

above the length of the chain in streptococci is largely determined by artificial conditions. V. Lingelsheim's *Streptococcus longus* is in general hemolytic and the term is practically identical with *Streptococcus pyogenes* or *Streptococcus hemolyticus*. The term *Streptococcus conglomeratus* was introduced by Kurth[22] to indicate those strains in which the elements were in the form of long chains wound together in a tangled mass and the term *Streptococcus longissimus* by Thalmann[23] for a long-chained streptococcus from the tonsils. Schottmüller,[9] on the basis of blood-laking properties and length of chains, made two main groups of pyogenic streptococci:

A. *Streptococcus hemolyticus* or *longus*. This form appears in long chains and produces clear zones of hemolysis about colonies on blood agar plates and a gradual clearing of blood agar in the immediate vicinity of the growth on agar slants.

B. *Streptococcus viridans* or *mitior*. This form appears in shorter chains and its colonies on blood agar are surrounded by green areas of discoloration.

Schottmüller's classification has been widely adopted. It possesses the distinct advantage that pathogenic action can be correlated to some extent with cultural features. *Streptococcus hemolyticus* is usually virulent and is frequently isolated from septicemias. *Streptococcus viridans* is in general less virulent and is encountered in chronic infections, especially in localized inflammations of the serous membranes.

More recently Smith and Brown[24] have made a very detailed study of hemolysis on blood agar plates. They differentiate three types of streptococci on horse blood agar, usually read at the end of forty-eight hours.

*Alpha Type.*—This form produces a zone of greenish discoloration and partial hemolysis about the colony, 1 to 2 mm. in diameter. About this is a second clearer partially hemolyzed zone.

*Beta Type.*—This form produces completely hemolyzed clear colorless zones about the colonies, usually 2 to 4 mm. in size.

*Gamma Type.*—These organisms grow on blood agar without perceptible hemolysis.

A number of other classifications of streptococci have been made by the use of various carbohydrates. The pioneer in this work was Gordon[25] who found that 300 strains from the saliva of normal individuals gave acid with dextrose, saccharose and lactose, rarely with raffinose and salicin, never with mannite; 300 strains from feces fermented dextrose, saccharose, and salicin regularly, frequently lactose, rarely raffinose and mannite; 200 strains from pathological conditions fermented dextrose, saccharose, and lactose, rarely other carbohydrates.

Gordon was followed by Andrews and Horder[26] who made six groups as follows:

1. *Streptococcus pyogenes*. Ferments saccharose, lactose, and salicin but does not coagulate milk. Usually grows in long chains.
2. *Streptococcus milis*. Like *Streptococcus pyogenes* but grows in short chains.
3. *Streptococcus anginosus*. Like *Streptococcus pyogenes*, but coagulates milk.
4. *Streptococcus salivarius*. Ferments dextrose, saccharose, lactose, raffinose and coagulates milk. Grows in short chains.
5. *Streptococcus fæcalis*. Ferments, dextrose, saccharose, lactose, and mannite. Grows in short chains.
6. *Streptococcus equinus*. Ferments dextrose but not lactose. Grows in short chains.

This classification permits a certain degree of correlation between pathogenic properties and cultural reactions. Thus the streptococci pathogenic to man belong to *Streptococcus pyogenes*, the non-pathogenic mouth streptococci to *Streptococcus milis* and *Streptococcus salivarius*, the streptococci found in scarlet fever to *Streptococcus anginosus;* the intestinal streptococci to *Streptococcus fæcalis* and the non-pathogenic streptococci from horse dung to *Streptococcus equinus*.

The most elaborate and in many respects the most satisfactory classification which has been suggested is that of Holman[27] who examined a very large number of streptococci from a great variety of sources.

Holman uses hemolysis and fermentation of lactose, mannite, and salicin as differential characters since all streptococci ferment dextrose. He makes 16 main types as follows:

1. *Streptococcus infrequens*. Hemolytic. Ferments lactose, mannite, and salicin.
2. *Streptococcus hemolyticus*. Hemolytic. Ferments lactose and mannite, but not salicin.
3. *Streptococcus pyogenes*. Hemolytic. Ferments lactose and salicin, but not mannite.
4. *Streptococcus anginosus*. Hemolytic. Ferments lactose, but not salicin or mannite.
5. *Streptococcus hemolyticus* II. Hemolytic. Ferments mannite and salicin, but not lactose.
6. *Streptococcus hemolyticus* III. Hemolytic. Ferments mannite, but not salicin or lactose.
7. *Streptococcus equi*. Hemolytic. Ferments salicin, but not mannite or lactose.
8. *Streptococcus subacidus*. Hemolytic. Does not ferment lactose, mannite, or salicin.
9. *Streptococcus fæcalis*. Non-hemolytic. Ferments lactose, mannite, and salicin.
10. *Streptococcus non-hemolyticus* I. Non-hemolytic. Ferments lactose and mannite, but not salicin.
11. *Streptococcus milis*. Non-hemolytic. Ferments lactose and salicin, but not mannite.
12. *Streptococcus salivarius*. Non-hemolytic. Ferments lactose, not mannite or salicin.

13. *Streptococcus non-hemolyticus* II. Non-hemolytic. Ferments mannite and salicin, but not lactose.
14. *Streptococcus non-hemolyticus* III. Non-hemolytic. Ferments mannite, but not salicin or lactose.
15. *Streptococcus equinus*. Non-hemolytic. Ferments salicin, but not mannite or lactose.
16. *Streptococcus ignavus*. Non-hemolytic. Does not ferment lactose, mannite, or salicin.

To a considerable extent Holman was able to link up pathogenic properties and source with cultural reactions. His classification is at present the only one based upon cultural characters which has practical value. Occasionally streptococci are found which do not fit in, but the types which Holman has worked out seem to be definite.

**Vaccines.**—Animals (rabbits) can be immunized against streptococci by killed cultures, attenuated cultures, etc. Their serum contains agglutinins for the strain employed and for closely related strains. This immune serum also has protective properties and has been employed from time to time in streptococcus infections in man. Thus far the results are not especially encouraging.

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## The Relation of Streptococci to Scarlet Fever

Of especial importance is the etiological relationship of streptococci to scarlet fever. For many years it has been known that streptococci are always present in the throats of scarlet fever cases, both in the severe type with extensive membrane formation and in the mild cases with comparatively little throat involvement. The majority of the complications also, like otitis media, endocarditis and pericarditis are due to streptococci. It has now been shown by George F. Dick and Gladys Henry Dick[1] that certain of the streptococci from cases of scarlet fever are capable of producing the disease in man. In several instances characteristic attacks of sore throat and fever, with a typical rash followed by desquamation, have followed swabbing of the throat with cultures of the organisms. Both mannite-fermenting and non-mannite-fermenting streptococci produce these lesions and symptoms. The Dicks[2] have further shown that filtrates of cultures of the streptococci which can produce the disease contain a toxin which causes an inflammatory reaction on intracutaneous injection in susceptible individuals. This is known as the "Dick test." It is carried out with Berkefeld filtrates from blood agar slant cultures (10 per cent. sheep blood), diluted 1 : 1000 in sterile salt solution, 0.1 c.c. of the filtrate being injected intracutaneously. This is known as the skin test dose. A positive reaction consists of a small circular area of erythema which appears in one to four hours. This reaches its maximum size in eighteen to thirty-six hours and then subsides in forty-eight hours. The erythema is sometimes accompanied by swelling and tenderness. An area of erythema 1.5 to 3 cm. in diameter is regarded as a positive reaction and in

some individuals an area 6 to 7 cm. in diameter occurs. This may be swollen with sharply raised edges. According to the Dicks this reaction was positive in over 40 per cent. (41.6 per cent.) of cases with no history of scarlet fever. The toxin can also be obtained by growing the organisms in plain broth containing 10 per cent. defibrinated sheep blood and filtering through a Berkefeld N or W.[3]

Prophylactic immunization with the streptococcus toxin has now been carried out in a number of persons with gratifying results.[4] The toxin of the streptococcus is inoculated at five-day intervals, the dosage increasing from 300 skin-test doses to 1000 skin-test doses. In 125 people who were immunized, including 73 nurses and 52 children with positive skin tests, no cases of scarlet fever subsequently developed.

Zinger[5] has found that the toxin of the streptococcus can be obtained from cultures of the streptococci in horse blood broth. When injected it has a local action on the capillary vessels of the skin and thus the Dick test differs from the Schick test in diphtheria where the toxic action is on the epidermal cells.

**Antitoxins for Scarlet Fever.**—Antitoxic sera for scarlet fever were first prepared by Moser[6] and by Moser and von Pirquet[7] by the immunization of horses with hemolytic streptococci from cases of the disease. Subsequently Dochez, Avery, and Lancefield[8] developed methods of typing streptococci by biological reactions and concluded that the streptococci from scarlatina form a specific type differing from other hemolytic streptococci. With these organisms Dochez[9] has produced transient fever and diffuse erythema followed by desquamation in a number of animals—dogs, hogs, and guinea-pigs. Dochez and Sherman[10] have immunized horses with these streptococci by introducing liquid agar into the cellular tissue of the neck and then inoculating the agar with streptococci. The immune horse serum produces a local disappearance of the rash on subcutaneous or intracutaneous injection in scarlet fever cases. This reaction is like the blanching of the skin described by Schultz and Charlton[11] from the administration of convalescent scarlet fever serum. Mair[12] has shown that the convalescent serum produces the blanching by virtue of its content in antitoxic substances which neutralize the toxic substances of the streptococci which are producing the rash. The antitoxic serum made by Dochez has been employed with considerable success in scarlet fever. Thus Blake, Trask, and Lynch[13] have given it in 12 early cases with complete recovery in 5. The serum was given in large quantities, 40 to 60 c.c., by intramuscular injection.

Antitoxins for scarlet fever have also been prepared by the Dicks,[14] sterile filtrates from the streptococci producing scarlet fever being employed for the immunization of horses. Neutralizing bodies were found in the horses' blood, 10 c.c. neutralizing 20 times the amount of toxin which produces fever, nausea, vomiting, general malaise, and scarlatinal rash in susceptible persons. This antitoxic serum has now been used with favorable results in prophylactic passive immunization. Subsequently the Dicks[15] have produced an antitoxin in which at least 1 c.c. neutralizes 1000 skin-test doses, a skin-test dose being the amount of toxin which causes a general reaction in susceptibles. When this serum is given by intramuscular injection it blanches the skin, lowers the temperature, improves the general condition, and lessens the incidence of complications and sequelæ.

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### STREPTOCOCCUS PYOGENES BOVIS Glage

Streptococci occur in a great many types of lesions in cattle, especially in mastitis, and are differentiated with great difficulty from streptococci found in man. Glage reports that they are somewhat smaller than the streptococci in man and form long chains in fluid media. Other reactions are like those of

the usual streptococci. They are not pathogenic for guinea-pigs by subcutaneous and intraperitoneal inoculation, nor for rabbits by subcutaneous, intraperitoneal, or intravenous administration.

Moore has found that some of the bovine streptococci produce septicemia in rabbits by intravenous inoculation. A streptococcus isolated from "Galtseuche" of cattle, *Streptococcus agalactiæ contagiosae bovis*, may be a specific organism for this disease which is contagious from animal to animal.

Glage: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, vi, 145.

### Streptococcus equi Schütz

Originally seen by Rivolta in 1873 in the pus from the glands in the disease of horses known as strangles (*Coryza contagiosa equorum*). Cultivated and described independently about the same time by Schütz.[1] Sand and Jensen,[2] and Poels. Description from Bongert.[3]

Fig. 56.—A bovine streptococcus, twenty-four-hour broth culture, stained by Gram.

**Morphology.**—In the pus individual cocci, diplococci, short chains and long chains made up of many elements lying between the pus cells. The cocci are round, oval, or oblong, almost bacillary. Frequently some of the individuals in the chain are much enlarged and stain deeply (arthrospores of Schütz). Sometimes whole chains are found made up of these large cocci. On staining with methylene-blue these large forms are seem to be tetrads and sometimes small chains may be seen to take their origin from these large forms, as if a chain made up of tetrads divided into two chains of small streptococci. In impression coverslips from agar colonies the organism appears as a tetrad (tetracoccus) on the periphery and as a staphylococcus in the center.

*Note.*—This formation of chains of small cocci from chains of large forms occurs in the streptococcus of cerebrospinal meningitis of horses (Bornasche Krankheit) and rarely in *Streptococcus pyogenes*. It may be found also in certain sarcinas.

**Staining Reactions.**—Stains very irregularly with ordinary anilin dyes, some individuals staining deeply and some lightly. The large elements usually take the stain intensely. With Loeffler's methylene-blue these large elements appear reddish-blue. Stains well with carbol-

thionin. Gram-positive, but not strongly so. Can be decolorized by long exposure to alcohol (over three-quarters of a minute).

**Cultivated** only at body temperature and on slightly alkaline media. No growth on media acid to phenolphthalein. According to Laabs the best reaction is produced by adding 1 to 3 per cent. normal sodium hydrate beyond the neutral to phenolphthalein point. Grows well on solidified horse serum (less well on beef serum), in serum broth, glucose broth, in sugar agar, in stab cultures and in serum agar.

Dies out easily under artificial conditions, kept alive best in serum agar stabs.

*Colonies* on *agar* flat, bluish, transparent, sharply contoured with a dark center and gray transparent veil-like periphery, 2 to 4 mm. in diameter. White flocculent sediment in water of condensation. Glycerin agar shows the same type of growth, small bluish-gray, viscid drops coalescing to a uniform expansion.

*Agar Stab.*—In twenty-four hours an abundant grayish-white streak which sends out peculiar rounded wing-shaped processes 3 to 4 mm. long. On the surface a small, flat, viscid drop. These radial prolongations into the agar are regarded by Sand and Jensen as a mark of differentiation from other streptococci. They do not always occur.

Hemolysis on blood agar plates and in blood broth.

*Gelatin* is a poor medium for this organism. In stabs after three to five days small white colonies appear, pin-head in size. Often no growth. No liquefaction.

*Coagulated Serum.*—Gray, glassy, irregular, drop-like colonies which coalesce to a viscid slimy mass. Flocculent opalescent turbidity in water of condensation. The slimy mass drys in a few days to a thin, irregularly fissured expansion.

*Broth.*—White, flocculent sediment and diffuse turbidity. More abundant growth in dextrose broth.

*Serum-broth.*—Thick, viscid, slimy precipitates with consistency like pus. Microscopically made up of long chains.

*Milk.*—Good growth. No change in appearance or reaction.

*Potato.*—Non-visible growth on surface with development in the substance which becomes grayish white (Sand and Jensen).

**Pathogenic Action.**—Pathogenic to gray and white mice which die of septicemia in about three days after subcutaneous inoculation or of pyemia after ten days. At autopsy the spleen is enlarged, multiple abscesses are found in the kidneys and spleen, rarely in the lungs. The splenic pulp always shows long chains which differentiate it from the streptococcus of horse pneumonia (Schütz streptococcus) which always appears as a diplococcus.

Field mice, guinea-pigs, and rabbits are but slightly sensitive to this organism. Rabbits develop an erysipelatous inflammation from subcutaneous injections on the ear and die from intraperitoneal inoculation of large doses. By the injection of pure cultures in the nasal mucosa of young horses Schütz, Sand and Jensen, and Poels have produced characteristic attacks of strangles. Cattle, sheep, swine, dogs, and birds are refractory. Animals can be actively immunized but the immune sera have no very definite protective properties.

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### STREPTOCOCCUS LACTICUS Günther and Thierfelder

Originally described by Günther and Thierfelder[1] and by Leichmann[2] in sour milk and regarded as a short bacillus. Named *Bacterium Güntheri* by Lehmann and Neumann[3] and *Bacillus lacticus* by Kruse.[4] First clearly recognized as a streptococcus apparently by Heinemann,[5] at least in this country. Morphologically and culturally like *Streptococcus pyogenes*, but usually devoid of pathogenic and hemolytic properties.

According to Salter[6] the streptococci normally present in milk, presumably of bovine origin, are characterized by their greatly diversified morphology, by their retention of Gram's stain, by the production of a high acidity and an early clotting in milk, and by the ability to withstand temperatures of 64° to 65° C. for half an hour. This resistance of the lactic acid streptococci to high temperatures is a matter of great practical importance in the problem of milk pasteurization, since it will permit pasteurization at temperatures high enough to kill off virulent human strains of streptococci and leave behind non-virulent bovine strains which will still clot milk normally after heating. It has also been shown by Ayers and Johnson[7] that many samples of milk contain streptococci which resist a temperature of 65° C. It should be noted in this connection that Beijerinck has found a more or less regular succession of races or strains of streptococci in milk differing in their resistance to acid, and believes that various strains develop as the acidity reaches a point favorable to them.

It has recently been shown by Langwill[8] that the acids produced by milk streptococci are the same as those formed by streptococci from other sources, although there is considerable difference in the rapidity with which they are formed.

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Fig. 57.—*Streptococcus lacticus* from twenty-four-hour culture on blood agar, stained by Gram.

### STREPTOCOCCUS COLI GRACILIS Escherich

Found by Escherich as a constant inhabitant of the intestinal tract in children fed on meat, and in meconium, but not in children during the period of suckling. In fresh gelatin cultures it appears as long S-like chains of 6 to 20 individuals. The size of the single coccus is 0.2 to 0.4 $\mu$, but can be much greater just before division. The chains on agar are shorter and do not appear on potato.

The organisms stain irregularly, some more deeply than others.

On *gelatin plates* the colonies are small, sharply contoured, at first round, later jagged or irregular, with a wide zone of liquefaction, the edge of which gradually disappears in the gelatin. Under high magnification delicate short extensions of the growth into the gelatin can be made out. The colonies are white or glistening in reflected light.

In *gelatin stab* a stocking-shaped liquefaction appears on the second day along the entire line of inoculation. The liquid gelatin shows a delicate whitish turbidity with small flat white colonies on the bottom. In eight to ten days the liquefaction reaches the edge of the glass and later is complete. In old cultures one finds a small quantity of finely granular sediment at the bottom of the glass and above it a clear fluid of an acid reaction.

Growth on *agar* is scanty, a delicate superficial colony.

*Blood-serum* not liquefied, growth in form of small glistening scales.

On *potato* the growth consists of a flat expansion with a frosted surface made up of small white button-like masses.

*Milk* slowly acidified and clotted. Cultures several months old show a brown or blackish coloration of the serum and of the casein, which is greatly lessened in amount.

*Non-pathogenic* to rabbits and guinea-pigs.

Escherich: Die Darmbakterien des Säuglings. 1886, p. 77.

### STREPTOCOCCUS COLI BREVIS Escherich

Found by Escherich in the meconium and in the stools of milk-fed children. Also present in large numbers in plates made from raw milk. Resembles *Streptococcus coli gracilis*. From fresh gelatin cultures it shows a number of small chains, which usually do not have more than 3 to 8 elements. Average size of each coccus 0.2 to 0.4 $\mu$. It varies greatly in size and often swollen intensely stained forms appear, twice as large as normal. Frequently such a megacoccus is found in the course of a chain or as the middle point of a small group.

In old cultures and on other media the chain formation can not be made out.

On *gelatin plates* the colonies have sharp round contours and form wide flat funnels of liquefaction containing clear, not turbid gelatin. The central colonies are very dark and olive green in reflected light.

In *gelatin stabs* the liquefaction is seldom stocking-shaped, usually it proceeds horizontally from above downward. The liquid gelatin is turbid with white flocculent masses of bacteria at the bottom. After ten to twelve days the gelatin is entirely liquefied with a small quantity of finely granular sediment. This originally has a dirty-white color, later a clear citron-yellow color, usually after several weeks. Growth on *agar* thin and greenish-yellow, on *blood-serum* a luxuriant citron-yellow ridge, not liquefying, and on *young potato* a flat expansion of the same color with a glistening surface.

Escherich: Die Darmbakterien des Säuglings. 1886, p. 86.

### STREPTOCOCCUS ENTERITIDIS Escherich

This name was given by Escherich[1] to streptococci described by Hirsh[2] and by Libman.[3] Hirsh's organism came from a fatal case of summer diarrhea. Microscopic examination of the stools showed almost pure streptococci in the mucopurulent masses. They were easily demonstrated by Weigert's method. They appeared as single cocci, diplococci, and chains of 3 to 20 elements. In cultures only short chains were formed. Division transverse, the two adjacent cocci being flattened on their opposing surfaces. In bouillon usually in diplococcus form. No capsules. Morphologically like Kurth's *Streptococcus involutus*.

**Cultivated** with difficulty except in sugar bouillon. No growth on *agar plates* visible microscopically. Under low power small, coarsely granular light brown colonies, visible in twenty-four hours at 37° C. Growth very slow in *agar stabs* both in the depths and on the surface, without spreading from line of inoculation.

In *sugar bouillon* a manifest turbidity in twelve hours at 37° C., a sediment in twenty-four hours. Reaction acid. Less rapid growth in bouillon without sugar, and in a mixture of bouillon and blood-serum (2 to 1). No gas in *sugar bouillon* or in *milk*.

**Virulent** to white mice by subcutaneous inoculation, the animals dying of septicemia and occasionally showing bloody diarrhea.

Not virulent to guinea-pigs or hares.

The same organism was found by Libman in one fatal case and in one case which recovered. Stained with fuchsin the size was 0.75 to 0.9 $\mu$, larger cocci often appearing in some of the chains. Cultivated on *gelatin slants*, fine white ridges appearing in twenty-four hours and showing a trace of liquefaction later. In *gelatin stab* discrete transparent colonies appeared along the line of inoculation, but no surface growth. Colonies on *gelatin plates* grew slowly, being barely visible in forty-eight hours, about 0.1 mm. in size. Microscopically they appeared as small light yellow points and disks of irregular form, the middle being dark and granular. On *agar plates* the superficial colonies were irregular ovals, with occasional

coarse granulations, fine jagged lighter peripheries, and darker yellowish middles. Maximum development in twenty-four hours, colonies visible as fine points. On *potato* the growth was a moist glistening thin expansion, made up of small white button-like masses. *Milk* acid in twenty-four hours, clotted in four days. On *agar* containing a little *human blood-serum* an abundant white growth developed in twenty-four hours, and transfers from this grew readily on all media. A similar increase of growth energy followed cultivation in fluid human blood-serum.

**Pathogenic** to mice by feeding, the animals developing bloody diarrhea and dying of septicemia.

Streptococci, probably of various species, were also found in fatal cases of summer diarrhea by Booker[4] and by Tissier.[5]

1. Escherich: Wien. klin. Wchnschr., 1897, x, 917.
2. Hirsh: Centralbl. f. Bakteriol. [etc.], 1897, xxii, 369.
3. Libman: Centralbl. f. Bakteriol. [etc.], 1897, xxii, 376.
4. Booker: Johns Hopkins Hosp. Rep., 1897, vi, 159.
5. Tissier: Recherches sur la flore intestinale des nourrissons. Paris, 1900, p. 55.

### STREPTOCOCCUS LIQUEFACIENS Sternberg

Originally obtained by Sternberg in the liver of yellow fever cadavers and in the human intestine. Present description from an isolation from the dejecta.

Fig. 58.—*Streptococcus liquefaciens* from twenty-four-hour culture on agar, stained by Gram.

**Morphology.**—Small cocci about 0.4 to 0.5 $\mu$ in diameter in diplococci, short chains, and bunches. Somewhat enlarged on dextrose agar, oval and pointed.

**Staining Reactions.**—Stains well with the usual anilin dyes. Gram-positive.

**Cultivated** without difficulty on the usual media. Grows best at 37° C. very slowly at room temperature.

*Plain Agar Plates.*—Surface colonies appear in twenty-four to forty-eight hours, small and white, $\frac{1}{2}$ to 1 mm. in size. Under low power round, regular, granular, opaque, with entire edges. Deep colonies pin-point, under low power oval and disk-shaped. At the end of four to five days the surface colonies are slightly larger, up to $\frac{1}{2}$ mm. in size, while after ten to eleven days they are 2 to $2\frac{1}{2}$ mm., delicate and white.

*Plain Agar Slant.*—Faint, delicate, whitish, non-spreading growth in twenty-four hours. After four to five days the growth is still delicate, much like that of *Streptococcus pyogenes.*

*Plain Agar Stab.*—Delicate line and slight surface growth in twenty-four hours. After

ten to twelve days the line growth is somewhat thicker and the surface growth slightly more abundant.

*Dextrose Agar Plates.*—Surface colonies small, delicate, whitish, about ½ mm. in size in twenty-four hours. Under low power granular, nucleated, with entire margins. Deep colonies pin-point, under low power ovals and disks. After four to five days the colonies on the surface may be 1 to 2 mm. in diameter. The agar gradually becomes acid.

*Dextrose Agar Slant.*—Faint delicate whitish growth in twenty-four hours, somewhat thicker and more opaque in four to five days. Agar highly acid.

*Gelatin Plates.*—Small pin-point colonies in forty-eight hours, under low power round, regular, uniform, margins entire or slightly irregular. Liquefaction begins early and in three to four days well-marked zones appear about the individual colonies. After six to seven days the gelatin may be entirely liquefied.

*Gelatin Stab.*—Faint line growth and slight surface growth in twenty-four hours. By the end of forty-eight hours there is a very distinct surface cupping which advances to a cup-shaped liquefaction in four to five days. The gelatin may be completely liquefied in six to seven days.

*Blood-serum.*—Faint, delicate, whitish growth in twenty-four hours, never becoming abundant.

*Potato.*—Very faint, white, dry, non-spreading growth.

*Broth.*—Turbidity and faint sediment in twenty-four hours. No scum. Both turbidity and sediment remain scanty, even in old cultures.

*Peptone.*—Faint turbidity and sediment. No scum.

*Litmus Milk.*—Acidification in twenty-four hours with a soft coagulum in forty-eight hours. The clot is slowly digested to a pinkish-red fluid.

*Fermentative Tubes:*

*Dextrose.*—Turbidity in bowl and arm. No scum. Some granular sediment. Highly acid.

*Saccharose.*—Appearance the same. Highly acid.

*Lactose.*—Appearance the same. Highly acid.

Not pathogenic to rabbits and guinea-pigs.

Sternberg: Manual of Bacteriology, 1893, p. 613.

### STREPTOCOCCUS SEPTICUS (Babes) Migula

Reported by Babes[1] as *Streptococcus septicus liquefaciens* from an autopsy in a child dead of putrid bronchiectasis, swelling of the spleen, parenchymatous degeneration of the kidneys and multiple arthritis. Organisms present in all the organs. Description from Cornil and Babes.[2] Named by Migula.[3] Coccus 0.4 $\mu$ in diameter in short chains. Gram-positive.

On agar at 36° C. small round transparent colonies, superficial and deep, the superficial colonies being more abundant than with *Streptococcus pyogenes.*

*Gelatin stab* at 20° C. Grayish striation producing a surface depression in forty-eight hours. Later a dentated growth along line of inoculation.

*Beef Serum.*—Superficial barely visible culture.

Virulent for mice and rabbits. Extensive edema develops at site of inoculation and the animals die in three to five days.

According to Cornil and Babes the same organism was found by Escherich in a case of septicemia in an infant.

1. Babes: Bakteriologische Untersuchungen über septiche Prozesse des Kindesalters., 1889, p. 22.
2. Cornil and Babes: Les Bactéries, 1890, i, 472.
3. Migula: Page 27.

### STREPTOCOCCUS MASTITIDIS Migula

Described first by Nocard and Mollereau[1] in contagious mammitis of milch cows. Same as *Streptococcus mastitidis sporadicæ* Guillebeau and as *Streptococcus agalactiæ contagiosæ* Kitt.[2] Named apparently by Migula.[3] Present description from Nocard and Mollereau.

**Morphology.**—In the milk of the affected animals and in cultures the organisms appear in chains, often long and twisted.

**Stained** by ordinary anilin dyes. Gram-negative when treatment by alcohol is prolonged.

**Cultivated** easily on the usual media, aërobically and anaërobically. In *bouillon* made from meat of chickens, veal, pork, horse, etc., neutral or slightly alkaline, an abundant growth appears in twenty-four hours in the form of a light, whitish, opaque deposit. On the slightest movement this deposit is disseminated through the fluid in light flocculent masses. More abundant growth in bouillon to which 2 to 5 per cent. glucose, lactose, saccharose, mannite, and especially glycerin is added. The addition of NaCl or peptone makes bouillon unfavorable for the organism. The neutral or alkaline broth becomes markedly acid. No growth in acid media. If the acidity be neutralized by the addition of a little powdered chalk, an abundant growth may be obtained. In media containing glucose, 1 to 2½ per cent., the sugar disappears in five to six days, with lactose in nine to ten days. With 5 per cent. sugar it requires six weeks to two months for the sugar to disappear. The product of the action of the organism is lactic acid.

*Gelatin Stab.*—Fine, round, surface pellicle on the third day, not widely spread, with a slight turbidity along the line of inoculation. Later small, whitish colonies, opaque, granular, forming a thick line with dentated borders in the center of the gelatin. Very rarely an arborescent line growth.

Less abundant growth of the same character on *agar*. Surface inoculations on *gelatin-agar*, or *serum gelatin* give rise to small, round, translucent, bluish colonies which coalesce to a delicate pellicle with thicker and more opaque borders. No liquefaction.

*Gelatin plates* at 16° to 18° C. show colonies on the third to the fourth day either on the surface or in the depth. They form small, round, slightly granular masses, definitely circumscribed. At first transparent they assume a clear yellow tone which turns to a brown when viewed under low power. They never reach a large size nor liquefy the gelatin. *Milk* acidified and coagulated to a dirty, yellowish-white mass.

Injection of pure cultures into the mammary gland of cows and goats produces an inflammation like that seen in the natural disease. Not virulent to small animals, dogs, or rabbits by ingestion. Virulence rapidly lost on artificial cultivation.

1. Nocard and Mollereau: Ann. de l'Inst. Pasteur, 1887, i, 109.
2. Kitt: Bakterienkunde, 1893, p. 322.
3. Migula: p. 19.

### Gram-negative Streptococcus of Doléris and Bourges

Isolated by Doléris and Bourges from the pus of a pelvic abscess.

Cocci in pus isolated, in pairs, and in chains of 4 to 6 elements about the size of *Streptococcus pyogenes*. In cultures the organisms are usually oval, elongated, their largest diameter corresponding to the long axis of the chain. Chains also short. On potato the organism appears as a staphylococcus. Stains well by anilin dyes. Gram-negative.

In *gelatin* growth begins on the second day and on the third day appears as a cloudy grayish filament. On the next day a streak appears made up of small, punctiform, whitish, confluent colonies. No liquefaction.

*Agar Slant.*—Along line of inoculation abundant, white, round colonies larger than those of *Streptococcus pyogenes*, translucent, bluish by reflected light.

*Lactose Agar.*—Acidified in two to four days.

*Milk* coagulated.

*Bouillon.*—Abundant turbidity and flocculent sediment.

*Potato.*—Invisible growth in twenty-four hours. Later, a distinct whitish expansion. Non-pathogenic.

Doléris and Bourges: Compt. rend. Soc. de biol., 1893, 9. ser., v, 1051.

### Étienne's Gram-negative Streptococcus

Isolated by Étienne from a pseudomembranous angina.

In material from the membrane the organism appears as a very minute micrococcus, measuring about 0.6 $\mu$ in diameter, grouped in chains of 6 to 10 elements.

*Stains* easily by ordinary anilin dyes. Gram-negative. Growth on *agar* as whitish, extremely small punctuate colonies like points of needles, appearing in twenty-four hours. No change later. Slight turbidity in *broth* in twenty-four hours, clearing in three days with slight flocculent deposit. *Gelatin slant* shows small colonies like those on agar. *Gelatin stab* shows small spherical isolated colonies along line of puncture. No liquefaction. No change in *milk*. *Potato.*—Colorless, clear, hyaline, isolated colonies in four days. Cultures lose viability rapidly.

Non-pathogenic.

Étienne: Arch. de méd. expér. et d'anat. path., 1895, vii, 503.

### Gram-negative Streptococcus of Cottet and Tissier

First obtained by Cottet and Tissier[1] from cystitis and from the stools in infantile diarrhea. Found also by Tissier[2] in the intestinal dejecta of young infants.

**Morphology.**—Spherical cocci arranged as small diplococci in the stools. In broth it appears as a diplococcus or in chains of 8 to 10 elements. The elements are smaller than those of the ordinary streptococcus or of the *Streptococcus enteritidis* of Hirsh and Libman. Not more than $\frac{1}{2}$ $\mu$ in diameter. In old cultures irregular, larger, spherical or elongated. In old anaërobic cultures it forms chains with the elements frequently larger and rod-shaped.

**Staining Reactions.**—Stains well with the basic anilin dyes, but is always Gram-negative both in young and in old cultures.

*Destroyed* by boiling or at 60° C. in fifteen minutes.

*Survives* in cultures more than a month.

**Cultural Characters.**—Grows both aërobically and anaërobically.

*Plain Agar.*—Whitish, opalescent colonies appearing in twenty-four hours, more transparent and smaller than those of *Streptococcus pyogenes*. At the end of forty-eight hours colonies .4 to .6 mm. Under low power uniform, with smooth edges. No increase in size after three to four days.

*Wertheim's Agar.*—Colonies like those on ordinary agar, but thicker.

*Gelatin Plates.*—Very fine opalescent colonies appearing at the end of about four days. No liquefaction.

*Gelatin Stab.*—Fine points along line of inoculation.

Growth poor in gelatin.

*Broth.*—Turbidity followed by a clearing of the broth with the formation of sandy deposit made up of very fine floccules.

*Milk.*—Acidification in about six days with a gelatinous coagulation without retraction of the clot.

*Potato.*—No growth on ordinary potato or on glycerin potato.

*Deep Glucose Agar.*—Fine, regular, whitish, lenticular colonies throughout the entire tube. No gas.

1. Cottet and Tissier: Compt. rend. Soc. de biol., 1900, lii, 627.
2. Tissier: Recherches sur la flore intestinale des nourrissons. Paris, 1900, p. 53.

### Streptococcus of Vaginal Catarrh of Cattle von Ostertag

Found originally in the discharges in vaginal catarrh by von Ostertag[1] whose observations have been confirmed by a number of observers. The organisms are usually extracellular but may be inside the leukocytes. They are present only in the local lesions and do not get into the blood-stream. Heckelmann[2] finds them in the cystic-degenerated ovaries of infected animals. The organisms are not present in the vaginal secretions of healthy cows where usually *Staphylococcus pyogenes aureus* and *Bacillus coli communis* are common.

**Morphology.**—Small streptococci in short chains, 6 to 9 elements, surrounded by a delicate capsule which cannot be stained.

**Staining Reactions.**—Stains with the usual basic anilin dyes, especially well in pus and in sections with Loeffler's methylene-blue. Decolorized by Gram's method. Cultivated without difficulty on ordinary agar, glycerin agar, coagulated blood-serum, gelatin, and broth. Gelatin and blood-serum not liquefied. Diffuse turbidity in broth. Faint growth on blood-serum. No growth in fluid serum. Most favorable medium glycerin agar and urine agar. Slight growth on acid media. No coagulation of milk. No gas in glucose media. No indol. No $H_2S$.

No definite production of hemolysins (Hašak[3]).

Easily destroyed by disinfectants, 2 per cent. lysol or creolin solution in one minute, rapidly by sublimate in a dilution of 1–5000.

*Pathogenic Action.*—Not virulent for ordinary laboratory animals, mice, guinea-pigs, rabbits, rats, and pigeons. Inoculated into the genitalia of cows the organisms produce lesions like those in the natural disease (von Ostertag), chronic purulent catarrh. No lesions produced by introduction of the organism into the genitalia of female sheep, goats, swine, horses, guinea-pigs, or rabbits.

In the natural disease this streptococcus is found in association with *Staphylococcus pyogenes aureus* and *Bacillus coli communis*. The latter organisms alone and in combination do not produce the disease.

1. von Ostertag: Monatschr. f. prakt. Tierh., 1901, xii, 533.
2. Heckelmann: Jahresb. Veterinärberichte der beamt. Tierärzte Preussens für 1908, ii, Teil, 26.
3. Hašak: Tierärztl. Centralbl, 1910, 575; 1911, 39.

### Streptococcus of Infectious Abortion of Mares von Ostertag

Obtained by von Ostertag from the heart's blood, fluids of body cavity and intestinal contents of fetuses from aborting mares.

They are short chained, Gram-negative and difficult to cultivate. They grow best in serum broth, in the transudates in the chest cavities of the aborted fetuses and on serum agar. In the fluid media they produce a uniform turbidity which in a short time, forty-eight hours, sinks to the bottom. On serum agar they grow as an expansion barely visible to the naked eye, in serum agar stabs as a delicate thread from the top to the bottom of the tube. No growth in milk or gelatin. Moderate growth in peptone broth as a diffuse turbidity sinking to the bottom. Cultures die out after 4 to 5 generations.

*Non-pathogenic* to mice, guinea-pigs, and rabbits. In one instance von Ostertag produced an abortion in a mare twenty days after an intravenous injection of a broth culture. Easily destroyed by disinfectants, instantly by sublimate 1–1000 in one minute by ½ per cent. carbolic acid, creolin, or lysol solutions.

von Ostertag: Monatschr. f. prakt. Tierh., 1901, xii, 386.

## GENUS 5. STAPHYLOCOCCUS Rosenbach

Parasites. Cells in groups and short chains, very rarely in packets. *Generally stain by Gram.* On agar streak good growth, of white or orange color. Glucose, maltose, sucrose, and often lactose fermented with formation of moderate amount of acid. Gelatin often liquefied very actively.

Type species *Staphylococcus aureus* (Rosenbach).
" *albus* (Rosenbach).
" *epidermidis albus* Welch.
" *citreus* (Passet)
" *cereus flavus* Passet.
" *ascoformans* (Johne).
" *bovis* (Glage).
" *zymogenes* (MacCallum and Hastings).

### Staphylococcus aureus (Rosenbach)

Bacteria were observed in association with pyogenic infections in the very early days of bacteriology, the species now called *Staphylococcus aureus* being apparently first brought to cultivation in fluid media by Pasteur in 1880. It was first accurately described and named by Ogston in 1882[1] and first obtained in pure culture by Becker in 1883 (Neisser[2]). The observations of Rosenbach, who named it *Staphylococcus pyogenes aureus* in 1884,[3] indicated clearly its significance in wound infections and in osteomyelitis. Called *Micrococcus aureus* (Rosenbach) by Migula.

**Morphology.**—In pus small spherical organisms arranged as single cells, pairs, and bunches containing a very irregular number of individuals. Occasionally tetrads and short chains of 4 to 5 elements are present, but the latter seldom form a straight line like that of a streptococcus. The unstained organisms appear as spheres. Occasionally when faintly stained they look like half-spheres (Neisser). The size of the organism varies greatly, according to their age, the medium in which they are living and the methods by which they are stained, and individual cells vary greatly in size in the same preparation. They measure 0.7 to 0.9 $\mu$ in diameter according to Neisser, 0.5 to 1 $\mu$ according to Kocher and Tavel,[4] 1.2 $\mu$ according to

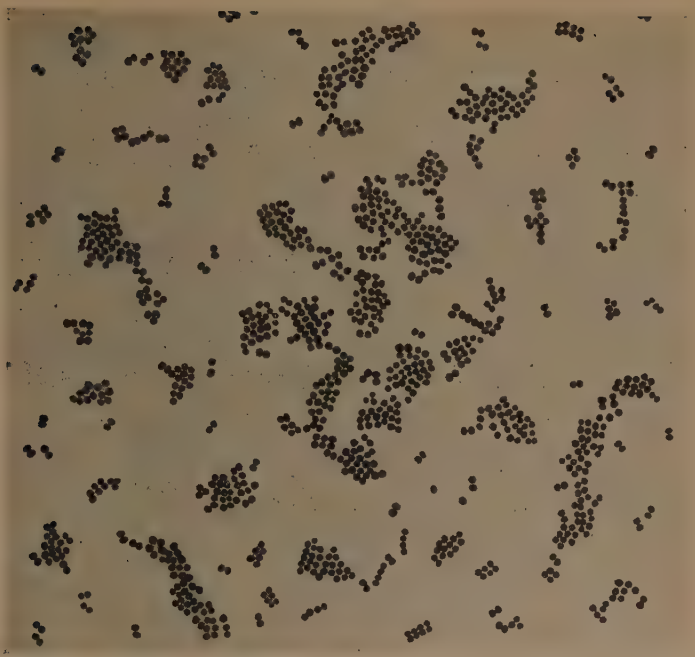

Fig. 59.—*Staphylococcus aureus* from twenty-four-hour culture on agar, stained with safranin.

Fig. 60.—*Staphylococcus aureus* from five weeks' culture, stained by Gram.

Macet. In the cultures examined by the present author the pus cocci are usually 0.5 to 0.6 $\mu$ in diameter in twenty-four-hour agar cultures stained by gentian-violet. In media containing sugar they are distinctly larger in twenty-four hours, many measuring 0.7 to 0.8 $\mu$. Others retain their usual size. They are generally larger in old cultures than in young and in very old cultures one can frequently find quite large almost giant-cells. They are larger in media with increased salt content and in slight acid or strong alkali. Nakanishi[6] believes that organisms from cultures two to three days old contain a minute spherical nucleus in the middle of the cell, but this is not generally accepted.

**Staining Reactions.**—Staphylococci may be stained easily by the usual anilin dyes, gentian-violet, carbolfuchsin, or methylene-blue. They stain well by Bismarck brown and aurantia (concentrated aqueous solution). They may be stained by hematoxylin (Lübbert, v. Lingelsheim, Pappenheim), but stain poorly by eosin. They retain Gram's stain both in pus and in cultures. Even in old cultures the majority of the organisms are Gram-positive. In the tissues they are best demonstrated by Weigert's or Nicolle's methods.

**Cultivated** without difficulty on the usual laboratory media, growing well at temperatures from 20° to 40° C. They also grow below 20° C., down to 6° to 8° C., and above 40° C. up to 42° to 43° C. They grow aërobically and anaërobically in an atmosphere of hydrogen, but not in an atmosphere of carbon dioxid, nitrogen, or illuminating gas. They grow in media

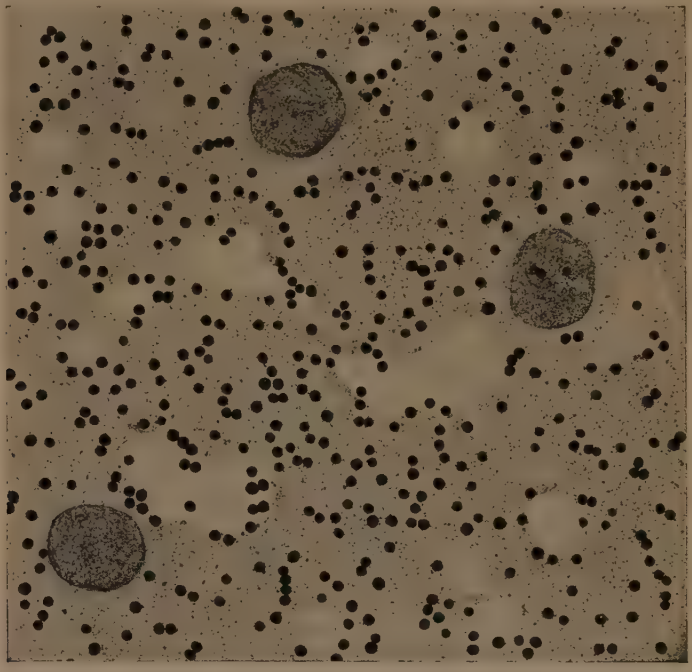

Fig. 61.—*Staphylococcus aureus* from rabbit kidney, stained by Gram.

of various salt concentrations up to 10 per cent. (Matzuschita). Best development in weak alkaline media. Development poor on acid media, an acidity of 30 c.c. normal acid to the liter inhibiting growth.

Winterberg[7] estimates that broth contains 5 to 80 million living organisms in twenty-four-hour cultures. Neisser states that 1 oesa of a twenty-hour broth culture contains 1 to 3 million organisms, 1 oesa of a twenty-hour agar culture 1000 million.

**Cultural Reactions.**—*Plain Agar Plates.*—Surface colonies in twenty-four hours $\frac{1}{2}$ to $1\frac{1}{2}$ mm. in size, slightly larger in forty-eight hours, $1\frac{1}{2}$ to 2 mm., yellowish. Under low power round, slightly irregular, opaque with entire edges. Deep colonies small, pin-point, under low power ovals and biconvex disks. The surface colonies increase in size and in four to five days are 2 to $2\frac{1}{2}$ mm. in diameter, golden-yellow in color. By ten or twelve days the colonies may be 3 to 4 mm. in size, especially when isolated, and the golden-yellow color is increased in intensity. Bottom colonies are usually colorless and spread widely. In old plates, two to three weeks, the isolated surface colonies may be 5 to 6 mm. in size and golden-yellow in color while the bottom colonies remain non-pigmented.

*Plain Agar Slant.*—Thick, moist, smooth, non-spreading golden-yellow growth in twenty-four hours. The growth increases slowly and in six to seven days is more abundant and deeper in color. Later the growth becomes heavier, more opaque, and distinctly golden-yellow.

*Plain Agar Stab.*—An abundant growth along the line of inoculation in twenty-four hours with a slightly spreading surface growth which is golden-yellow in color. The growth increases slowly and in six to seven days both line and surface growth are abundant. The surface growth has the typical golden-yellow appearance.

Rarely freshly isolated strains, especially from the blood or the tonsils, are not characteristic on agar. The growth is much less abundant in twenty-four hours and the culture has a slight resemblance to that of a streptococcus. By the end of forty-eight hours the colonies are increased in size, some pigment is formed, and the cultures rapidly become typical.

*Dextrose Litmus Agar Plates.*—Surface colonies in twenty-four hours round, regular, slightly yellow, 1 to 1½ mm. in diameter. Under low power opaque, granular, margins entire but rather thin. The centers are dense and may be nucleated. Deep colonies small, almost pin-point, under low power ovals and disks. Where the plates are crowded the agar is entirely acid. With isolated colonies zones of acid appear about them. The colonies increase fairly rapidly in size and in two to three days are 2 to 3 mm. in diameter. The color is a deep yellow, somewhat modified by the acid. Later all the plates turn acid and in seven to eight days the colonies may be 4 to 5 mm. in diameter, somewhat elevated, golden-yellow. The bottom colonies tend to spread and remain colorless.

*Dextrose Litmus Agar Slant.*—Thick, white, non-spreading growth in twenty-four hours, agar highly acid. Growth increases in size, usually remains whitish, developing later a peculiar yellowish-red due to the modification of the pigment by the acid. In old cultures the growth is thick reddish or yellowish-red, the agar remaining acid.

*Glycerin Agar.*—Growth like that on plain agar.

*Blood-serum.*—In twenty-four hours thick, dry, non-spreading golden-yellow growth. In three to four days the growth is characteristic, the yellow color increased in intensity. By eight to ten days the growth reaches its maximum as a golden-yellow expansion. No liquefaction.

*Blood Agar Plates* (human or rabbit blood).—Colonies large, opaque, frequently surrounded by a zone of hemolysis.

*Gelatin Plates.*—Colonies appear in forty-eight hours, small and rather delicate, under low power round, regular, uniform, yellowish, with entire margins. In three to four days colonies are somewhat larger, measuring ½ mm. in diameter, lying in zones of liquid gelatin 2 to 3 mm. in size. When the plates are crowded the gelatin may be entirely liquefied in three to four days. With isolated colonies the zones of liquefaction about the colonies increase and may be 1 cm. in size. Inside of these lie the colonies which now appear somewhat hazy and irregular.

*Gelatin Stab.*—Growth fairly rapid along the line of inoculation in twenty-four hours with some surface cupping. In forty-eight hours the growth is more abundant with a cup-shaped liquefaction on the surface, the liquid gelatin being turbid. In six to seven days the liquefaction is increased along the line of inoculation and on the surface, the liquid gelatin being yellowish. In twelve to fifteen days the gelatin may be entirely liquefied and appear turbid with flakes of yellow pigmented material floating about in it.

According to Neisser the optimum gelatin reaction for the staphylococcus is obtained by adding 0.68 c.c. normal sodium hydrate to each 100 c.c. of gelatin.

*Potato.*—Faint yellowish growth in twenty-four hours increasing fairly rapidly. By the end of five to six days it is fairly abundant and golden-yellow.

*Broth.*—Rapid growth, dense turbidity and faint sediment in twenty-four hours. Turbidity and sediment increased without scum formation. By the end of seven to eight days the turbidity is marked and the sediment golden-yellow. The broth does not clear and cannot be cleared by centrifugation except at very high speeds. According to Neisser broth cultures have a pasty odor. In broth containing rabbit's blood the blood is hemolyzed.

*Peptone.*—Moderate turbidity and faint sediment in twenty-four hours without scum. The turbidity and sediment are increased in three to four days and may become fairly abundant in seven to eight days. The sediment is apt to remain whitish.

*Litmus Milk.*—Growth rapid, cultures becoming acid in twenty-four hours and sometimes coagulated in two to three days. The coagulum is frequently soft and often does not appear except on heating the tubes.

*Carbohydrates.*—In dextrose fermentation tubes growth appears in twenty-four hours as a turbidity in the bulb without scum, the arm remaining clear. The growth gradually increases, the sediment becomes more marked and the turbidity extends up into the closed arm. Reaction highly acid at the end of three days. Similar reactions in saccharose and lactose which both become acid. In lactose a faint fragile scum frequently develops on the surface. Other media upon which the staphylococcus grows readily are the solid milk media of Raskin, coagulated blood-serum, glycerin serum agar, agar containing blood of man and various species of animals. Poor growth on Endo-agar. Moderate growth in alkaline urine, poor growth as a rule in acid urine.

**Resistance.**—Destroyed somewhat easily by heat. Killed at 52° to 53° C. in two to three hours (Ficker), at 62° C. in ten minutes, at 80° C. in one and a half minutes (Sternberg), at 60° C. in one-half to three-quarters of an hour, at 70° C. in five minutes (Neisser). Under all conditions killed at 70° to 80° C. in one-half hour. Resistant to low temperatures, not being killed by liquid air (Paul and Prall). Killed by sunlight in three to five hours. Resists drying. Dried on paper requires one hundred and ten days at room temperature for destruction, ninety days at 37° C. Survives five to six minutes on silk threads (Kirstein). Fairly resistant to ordinary disinfectants. Destroyed in ten minutes in 3 per cent. carbolic acid, 1 per cent. lysol, 4 per cent. lysoform. Destroyed by 50 to 55 per cent. alcohol in ten minutes, not destroyed by absolute alcohol. Destroyed easily by sublimate in salt sodium; at 15° C. in 1 per cent. solution, at 20° C. in $\frac{1}{2}$ per cent. solution, and at 25° C. in $\frac{1}{4}$ per cent. solution (Kruger). Destroyed by 2 per cent. lactic acid in three hours; by 1 per cent. in six hours (Menge and Krönig). Killed by 0.19 per cent. HCl in fifty minutes (Kobrhel). Most sensitive to oxalic acid (Abbott and Nuttall). Inhibited completely by formaldehyd in aqueous solution 1:5000; destroyed in 1 per cent. solution in fifty-five minutes, in 10 per cent. solution in ten minutes.

Destroyed by anilin dyes (Koch, Boering, and Boer). The inhibitory activity of methyl violet was observed by Beck in 1888, by Penzold[9] and Stilling[10] in 1890. Stilling found that methyl violet killed staphyloccoci in dilution 1 : 10,000 in five to ten minutes, in 1 : 25,000 in fifteen minutes, in 1: 50,000 in one hour. Inhibited by crystal violet (Drigalski and Conradi) 1 : 100,000 up to 1 : 1,000,000 (Nöggerath[11]). Also destroyed by methylene-blue and safranin in a 1 to 2 million solution.

Specially sensitive to gentian-violet. Thus Churchman[12] has found that *Staphylococcus aureus* and *Staphylococcus albus* are killed by gentian-violet in a dilution of 1: 1,000,000 in broth in forty -eight hours.

Dissolved by pyocyanase (Harrison).

**Pathogenic Action.**—Pure cultures of *Staphylococcus aureus* are virulent for the usual laboratory animals, but there is great difference in the strains. According to Neisser the virulence for animals runs parallel to the virulence for man. The lesions have been most carefully studied in rabbits. In strains of the usual virulence, $\frac{1}{10}$ c.c. of twenty-four-hour broth cultures injected intravenously kills rabbits of average size in four to six days. At autopsy small abscesses are present, especially in the heart muscle and in the kidney, but may be found in diverse locations, in the various joints, at the junction of the bone and cartilage of the ribs, in the vertebræ, and occasionally in the muscles, particularly in the psoas muscles. Abscesses occur rarely in the lungs, mediastinum, and pericardium. They do not occur in the liver. The bone changes resemble a true osteomyelitis. Infections of the spinal cord have been observed by Thoelet and Masselin, changes in ganglion cells of the brain by Sanders (Neisser). Rabbits often resist small dosage, however, and sometimes succumb after long periods. With more highly virulent staphylococci doses of this size are apt to produce a septicemia, the animals dying in twenty-four to forty-eight hours without abscess formation.

Subcutaneous inoculation in rabbits produces localized abscesses, but large doses are required, 1 c.c. of twenty-four-hour culture. By injuring the tissues by disinfectants (3 per cent. carbolic acid or 0.1 per cent. sublimate) abscesses are produced more easily (Duflacq). Abscesses may also be produced by direct injection into the muscles. The pus is always thick and caseous. In intraperitoneal inoculation large doses are required to produce peritonitis and death in rabbits. Other lesions produced in these animals include corneal opacity or perforating corneal ulcer after previous injury (Leber) and endocarditis.

After injuring the heart valves Orth[13] and Wyssokowitch[14] produced ulcerative endocarditis by intravenous inoculation. According to Ribbert and Jos. Koch this condition can be caused without previous injury to the valves.

In dogs small doses produce abscesses by subcutaneous inoculation. Römer has produced conjunctivitis on a conjunctiva injured by dust, etc., and Axenfeld has produced it in newborn puppies without previous injury.

In mice subcutaneous injection is usually without effect; with intraperitoneal inoculation the animals die in one to three days, pyemia not being observed at autopsy.

In large animals (horses and cattle) abscesses may be produced by subcutaneous injection.

Garrè[15] has produced a panaritium and a large furuncle by rubbing staphylococci into the human skin. Abscess, panaritium, and phlegmons were also produced by Schimmelbusch by subcutaneous injection.

The virulence of the organisms is increased by animal passage, the organisms being cultivated from the abscesses. By intramuscular inoculation v. Lingelsheim raised the virulence for rabbits from a fatal dose of 5 c.c. to one of $\frac{1}{100}$ c.c.

Living broth cultures of *Staphylococcus aureus* produce amyloid degeneration in rabbits, mice, and hens on subcutaneous injection of small quantities (0.25 to 0.3 c.c.), but not in guinea-pigs and cats (Davidsohn[16]). Filtrates injure the ganglion cells of the sympathetic nerves (Van de Velde), of the kidney cortex (Levaditi[17]), and the subcutaneous cellular tissue (v. Lingelsheim).

**Occurrence.**—Typical hemolyzing staphylococci occur rarely on the human skin, the staphylococci found there being usually non-pigmented or non-hemolyzing varieties. They are normally present in the mouth cavity and may be found in the saliva. They are rarely found on the normal conjunctiva (Römer), on the skin of the eyelids and ciliary edges. They are present occasionally in the intestine and may rarely be isolated from the stools. According to Amersbach[18] they may be present in the normal vagina, but this is rare (Menge and Krönig[19]). They are occasionally encountered in the male and female urethra. Outside the body typical staphylococci are rarely found, the majority of the pigmented cocci isolated being air organisms. They are occasionally encountered in the air of operating rooms and hospital wards (Binaghi). They have been found in factory dust (Arens[20]), in school room dust (Cacace[21]), in street dust (Matzuschita), and in the dust of street cars (Hoertl). Many of these isolations are non-pathogenic. Typical pathogenic staphylococci have been found in the air by Concornotti.[22] They are practically never present in ordinary water, but may be found in bath water.

Staphylococci have been found as the primary cause of many pathological processes, particularly those in which pus is produced, and are common in mixed infections and as secondary invaders. They are present in the majority of abscesses and furuncles connected with the skin, penetrating the skin through the hair-follicles or through breaks in the horny layer. They are the etiological agents in impetigo contagiosa and in sycosis. According to Jochmann a true erysipelatous inflammation may be due to staphylococci. They occasionally produce phlegmonous inflammations (E. Levy[23]), and a peculiar staphylomycosis, which may be fatal, has been described by Hallopeau.

In the mouth staphylococci have been regarded as the cause of aphthous inflammations of the mucous membrane of Jadassohn, and as the cause of exudative anginas, especially in children, by Fischl. Neisser believes that they can produce true diphtheria-like inflammations of the pharynx. They are the etiological agent in the majority of cases of osteomyelitis, in many cases of periostitis, but are apparently rare in primary infections of the joints. They are frequently present in lung abscesses and Weyl has reported a few cases (4) of true pneumonia due to staphylococcus. In appendicitis and peritonitis staphylococci are common, both alone and in association with other organisms, especially in those cases with fluid pus. They serve as the etiological agent in many cases of meningitis both of the brain and cord, especially in the cases following injury or surgical operation. Many cases of otitis media are due to staphylococci. They may be the cause of urethritis, especially following instrumentation, of cystitis, and of pyelonephritis. They are often found in phlebitis. Finally, staphylococci are found in many cases of empyema, in subphrenic abscesses, in brain abscesses after otitis media, in abscesses in the parotid gland, in liver abscesses, etc. They are the usual cause of pyemia following their discharge into the blood-stream from local infections, especially furuncles about the upper lips. Many cases of endocarditis and pericarditis are due to staphylococci and in some of the cases the organisms may be isolated from the blood-stream (Libman). Whether they serve as the primary agent in true septicemias with a multiplication of bacteria in the blood is by no means clear. In animals staphylococci are common in many types of suppuration. They have been found in horses by Lucet, in dogs by Lucet and Almy, in catarrhal inflammations of the udder in cattle by Jensen,[24] in osteomyelitis in young geese by Lucet, and in abscesses in birds by Karlinsky. It has not thus far been determined whether the staphylococci found in animals are the same as those in man.

**Chemical Reactions.**—*Staphylococcus aureus* takes up oxygen and gives off carbon dioxid in plain broth (Stagnitta-Balistrer). On media containing sulphur it produces hydrogen sulphid. It produces ammonia, reduces nitrates to nitrites, and does not form indol (Bayne-Jones). It reduces methylene-blue and Bordeau red. It ferments glucose with the formation of lactic acid, butyric acid, and formic acid. In 1 per cent. solution so much acid may be produced in twelve to fourteen days that the organisms die out (Smith). In peptone-free solutions sugar is said by Hugouneng and Doyon[25] to be converted into alcohol, especially under anaërobic conditions. Fats may be converted into fatty acids, and hippuric acid decomposed into benzoic acid and glycocol. Hucker states it cannot utilize ammonium salts as the sole source of nitrogen.

**Pigment Production.**—The pigment of *Staphylococcus aureus* is produced best in diffuse daylight and does not occur in direct sunlight. It is most abundant on certain media, especially potato and rice flour. Free oxygen is necessary and the most favorable temperature 20° to 22° C. Beijerinck places *Staphylococcus aureus* in the "chromophoren" bacteria which produce pigment as a valueless excretion. The pigment lies in dentritic crystals between the bacterial cells and does not diffuse into the media. It is a lipochrome (Zopf) or fat-like substance which gives the lipocyanin reaction with concentrated sulphuric acid. (The yellow pigment is here converted to blue crystals which give the aërolein reaction and which are deliquescent.) It is insoluble in water and soluble in alcohol, ether, carbon bisulphid, benzol, and chloroform. It gives a golden-yellow solution which is not altered by sulphuric, hydrochloric, or acetic acid, but gives a greenish-blue ring with nitric acid. The absorption bands in the spectrum lie at F and between F and G. The pigment is best obtained from the dried and pulverized organisms by treating them with acetic acid in the heat and then shaking them out with chloroform and ether.

**Ferments.**—*Staphylococcus aureus* produces a number of ferments of which the one dissolving gelatin is most easily demonstrated. There is some evidence of a proteolytic ferment. Thus a softening of Loeffler's blood-serum often occurs, especially if the organisms are cultivated in plates on mixtures of agar and serum. A clear zone develops at the point of inoculation. Eijkman[26] also has found a clear zone when the organism be planted on mixtures of milk and agar, caused by the solution of the milk proteins. Rietsch has found some fibrinolysis from precipitated cultures and believes that the organism produces a fibrin ferment. When the living organisms are allowed to act on protein in aqueous solution albumoses, traces of peptone, and ammonia are evolved. Emmerling[27] found amines and fatty acids also from protein decomposition. A fat-splitting ferment has been described by Fermi.[28]

Ferment production takes place best at 37° C. and is prevented by the addition of sugar. Auerbach believes this is due to some change in metabolism. A protein-sparing seems to occur in sugar solutions with this organism as with other species of bacteria.

**Leukocidin.**—If rabbits be injected with staphylococci into the pleural cavity the exudate has a destructive action on the rabbit leukocytes as first shown by Van de Velde.[29] The substance with this action is known as leucocidin. It is destroyed at 57° C. and is also present in the cultures of staphylococci, but only in pathogenic strains. Under its influence the leukocytes show a hyaline degeneration with the loss of the nucleus. The leukocytes of the rabbits are more sensitive than those of puppies, mice, and guinea-pigs. The leukocytes of the frog are resistant (v. Lingelsheim[30]). Neisser and Wechsberg[31] have shown that if methylene-blue be added to the mixtures of leukocytes and filtrates its reduction serves as a measure of the integrity of the cells. The cessation of reduction is a sign of the destruction of the leukocytes. In cultures four days old about .0075 c.c. of the filtrate was leucocidal. The substance is destroyed by heating to 50° C. for twenty minutes and cannot be reactivated.

**Hemolysin or Hemotoxin.**—*Staphylococcus aureus* produces a lytic substance for rabbit erythrocytes, as first definitely shown by Neisser and Wechsberg. It is present in broth cultures kept at 37° C. from about the fourth day and is most abundant in cultures nine to thirteen days old. It is inactivated at 56° C. and cannot be reactivated. This hemolysin can also be demonstrated on blood-agar plates. Kraus and Ludwig[32] report that the corpuscles are also agglutinated. Hemolysins are not produced by pigmented micrococci of environmental origin.

**Immunity Reactions.**—Agglutinins for the staphylococci are occasionally present in the blood of men suffering from infection with this organism, but not regularly (Silverstrini). In immunized rabbits agglutinins have been found in the blood-stream by Landsteiner,[33] and Kolle and Otto[34] have shown that these act upon pyogenic "aureus" and "albus," but not upon the yellow and white non-pathogenic environmental micrococci. Antihemolysins or antihemotoxins may be produced in rabbits and goats by subcutaneous and intravenous inoculation. These neutralize the lysins from all "aureus" and "albus" strains. Antileukocidins have not been produced artificially in animals. Vaccines from staphylococci have been used with success in certain types of infection, especially sycosis, furunculosis, etc.


1. Ogston: Brit. M. J., 1881, i, 369.
2. Neisser: Die Staphylococcen. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1912, iv, 355.
3. Rosenbach: Mikroorganismen bei den Wundinfections-Krankheiten des Menschen. Wiesbaden, 1884.
4. Kocher and Travel: Vorlesungen uber chirurgische Infectionskrankheiten. Basel, 1895.
5. Nakanishi: Centralbl. f. Bakteriol. [etc.], 1901, xxx, 97; 145; 193; 225.
6. Matzuschita: Ztschr. f. Hyg., 1900, xxxv, 495.
7. Winterberg: Ztschr. f. Hyg., 1898, xxix, 75.
8. Beck: Ztschr. f. Hyg., 1901, xxxvii, 294.
9. Penzold: Arch. f. exper. Path. u. Pharmakol., 1890, xxvi, 310.
10. Stilling: Anilinfarbstoffe als Antiseptica. Strassburg, 1890.
11. Nöggerath: Charité-Ann., 1911, xxxii. Therap. Monatshefte, 25. Jahrg., Aug. 1911.
12. Churchman: J. Exper. M., 1912, xvi, 221.
13. Orth: Virchow's Arch., 1886, ciii, 333.
14. Wyssokowitsch: Virchow's Arch., 1886, ciii, 301.
15. Garrè: Beitr. z. klin. Chir., 1893, x, 241.
16. Davidsohn: Virchow's Arch., 1897, cl, 16.
17. Levaditi: Congress Internat., 1900. Mastzellenleukocytose. Paris, 1902.
18. Amersbach: Ztschr. f. Geburtsh. u. Gynäk., 1912, lxx, 511.
19. Menge and Krönig: Bakt. weibl. Genitalkanal. Lepizig, 1897.
20. Arens: Arch. f. Hyg., 1894, xxi, 325.
21. Cacace: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1901, xxx, 653.
22. Concornotti: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1899, xxvi, 492.
23. Levy: Ueber die Mikroorganismen der Eiterung. Leipzig, 1891.
24. Jensen: Lubarsch-Ostertag Ergebnisse, 1899.
25. Hugouneng and Doyon: Ref. Maly's Jahresber., Bd., 28, 1898.

26. Eijkman: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1901, xxix, Orig.
27. Emmerling: Baumgarten's Jahresbericht, 1887.
28. Fermi: Centralbl. f. Bakteriol. [etc.], 1890, vii, 469.
29. Van de Velde: Cellule, 1894. Presse med., 1900.
30. v. Lingelsheim: Aetiologie u. Therapie der Staphy. Infekt. Berlin, Wien. 1900.
31. Neisser and Wechsberg: Ztschr. f. Hyg., 1901, xxxvi, 299.
32. Kraus and Ludwig: Wien. klin. Wchnschr., 1902, xv, 382.
33. Landsteiner: Wien. klin. Wchnschr., 1897, x, 439.
34. Kolle and Otto: Ztschr. f. Hyg., 1902, xli, 369.

### STAPHYLOCOCCUS ALBUS (Rosenbach)

Originally described as *Staphylococcus pyogenes albus* by Rosenbach[1] in association with *Staphylococcus aureus*. Called *Micrococcus pyogenes* (Rosenbach) by Migula.[2] An active discussion has always been held as to whether this is a definite species.

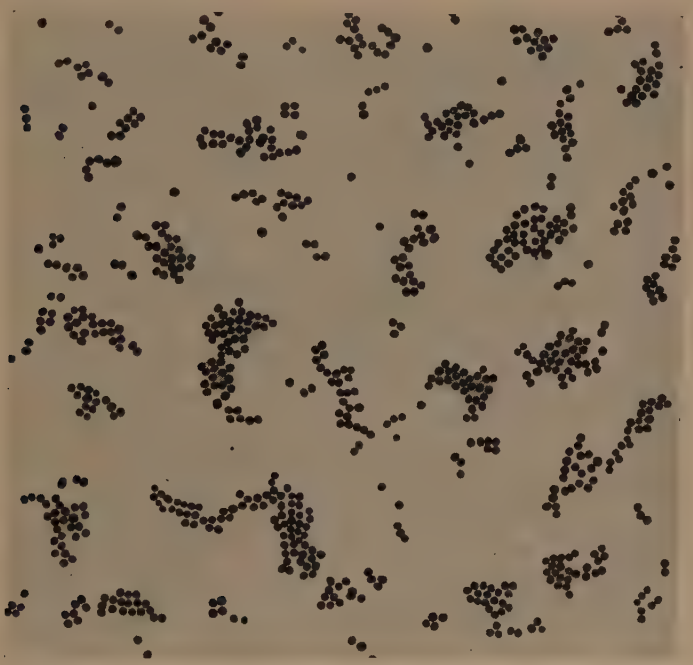

Fig. 62.—*Staphylococcus albus* from two weeks' culture, stained by Gram.

The cultural reactions and pyogenic properties are practically identical with those of *Staphylococcus aureus*. Many strains of "aureus" lose their pigment production on long laboratory cultivation and many strains regarded as non-pigmented originally are found to produce pigment later. According to Neisser,[3] who has possibly investigated the staphylococci with the greatest care, the cultural reaction and pathogenic properties, especially the hemolysin and leukocidin production, are the same for "albus" and "aureus." Occasionally white staphylococci are obtained from the skin and mucous membranes which are devoid of pathogenic action, but is not certain that these are true staphylococci. This species should be sharply differentiated from the white micrococci of environmental origin which are always non-pathogenic.

1. Rosenbach: l. c.
2. Migula: p. 87.
3. Neisser: Die Staphylococcen. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1912, iv, 355.

### STAPHYLOCOCCUS EPIDERMIDIS ALBUS Welch

Described by Welch from the deeper layers of the skin as an important cause of mild surgical infections, especially skin abscesses about sutures, etc. Growth scanty on artificial media. Slow liquefaction of gelatin and slow coagulation of milk with acid production. No pigment production.

Virulence for animals slight.

Welch: Am. J. M. Sc., 1891, cii, 439.

### STAPHYLOCOCCUS CITREUS (Passet)

Reported from human pus by Passet as *Staphylococcus pyogenes citreus*. It resembles *Staphylococcus aureus* in morphology and cultural reactions, but produces a citron-yellow pigment which is not identical with the golden-yellow pigment of "aureus."

Passet: Untersuchungen über die Aetiologie der eitrigen Phlegmone des Menschen. 1885, p. 9.

### STAPHYLOCOCCUS CEREUS FLAVUS Passet

Described by Passet from human pus but without pathogenic properties for animals. Now usually regarded as an environmental micrococcus. Not *Micrococcus flavus* (*Neisseria flava*), which is a Gram-negative facultative parasite described by v. Lingelsheim from the normal throat.

### STAPHYLOCOCCUS ASCOFORMANS (Johne)

Obtained by Johne[1] from suppurative lesions in horses. Same as *Micrococcus botryogenus* (Rabe[2]), *Botryococcus ascoformans* (Kitt[3]), and *Ascococcus Johnei* (Cohn).

Micrococci have been isolated by a number of observers from a disease-complex in horses resembling actinomycosis of cattle for which the name botryomycosis, proposed by Bollinger, is usually employed. The pathological process consists of new-formed connective tissue in which grayish or yellowish-red nodules appear, granulation tissue with soft centers. The larger nodules break down and form fistulas. The disease may occur also rarely, seldom in cattle and swine. It has been reported rarely in man (Galli-Valerio[4]).

From the interior of these nodules large micrococci 1 to 1.5 $\mu$ in diameter may be obtained. They lie in smaller or larger groups and may be stained by ordinary anilin dyes, best by anilin gentian-violet (Rabe). Good results are obtained by preliminary treatment with dilute acetic acid. Culturally and on animals the organisms correspond to *Staphylococcus pyrogenes aureus*.

1. Johne: Deutsche Ztschr. f. Thiermed., 1886, 204.
2. Rabe: Deutsche. Ztschr. f. Thiermed., 1886, 137.
3. Kitt: Centralbl. f. Bakteriol. [etc.], 1888, iii, 177.
4. Galli-Valerio: Arch. de parasitol., 1889, p. 77. Centralbl. f. Bakteriol. [etc.], 1902, xxxi, 508.

### STAPHYLOCOCCUS BOVIS (Glage)

Staphylococci occur in a great many suppurative lesions of cattle. Glage believes that these organisms can be differentiated from the staphylococci in man by their smaller size.

They grow sparsely on ordinary media and rapidly die out in cultures. The colonies on agar are delicate grayish and granular with jagged edges and develop little color. On agar slants a transparent moderate expansion develops with irregular edges, in agar stabs a line growth made up of punctiform colonies. Calf-meat broth shows a slight turbidity in twenty-four hours, followed by clearing and the formation of a grayish sediment which is not viscid. Liquefaction of gelatin does not occur.

**Pathogenic Action.**—Harmless for rabbits and guinea-pigs by any type of administration.

Glage: Die Eiterungen bei den Haustieren. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl., 1913, vi, 145.

### STAPHYLOCOCCUS ZYMOGENES (MacCallum and Hastings)

Described originally as *Micrococcus zymogenes* by MacCallum and Hastings[1] from the blood and organs of a patient dying of acute endocarditis. Also found by Harris in the contents of an old cess-pool and by Harris and Longcope[2] in 4 other autopsies.

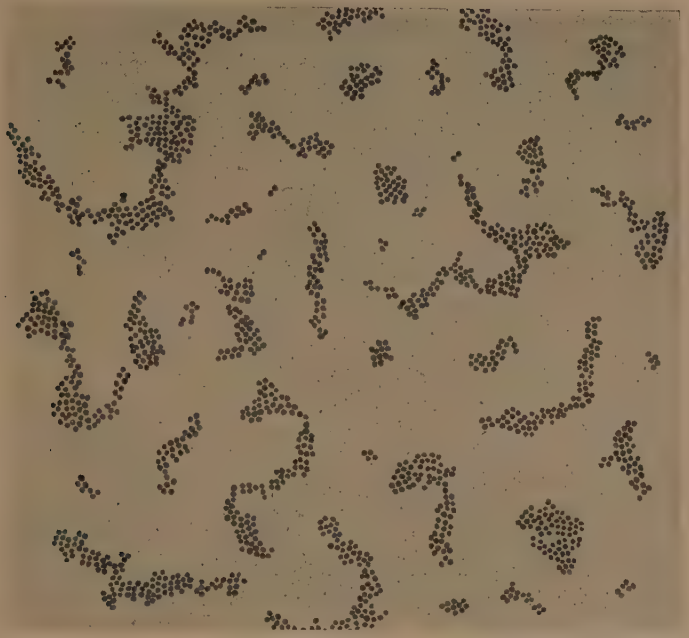

Fig. 63.—*Micrococcus zymogenes* from twenty-four-hour culture on plain agar, stained by Gram.

**Morphology.**—Very minute micrococcus, often elongated or elliptical in outline. Much smaller than *Micrococcus pneumoniæ*. Occurs singly, in pairs, in short chains, and in clumps. In broth cultures it may appear in chains of 20 or more elements. In the chains the long axis of the coccus is often transverse to that of the chain.

**Staining Reactions.**—Stains well with ordinary anilin dyes. Strongly Gram-positive. No capsule. Non-motile.

**Cultural Characters.**—*Micrococcus zymogenes* is a facultative anaërobe growing as luxuriantly in Buchner jars as in the air. Its growth is usually delicate and somewhat resembles that of the streptococci.

*Agar Slant.*—Profuse thin slightly elevated growth along the line of inoculation. Growth somewhat moist and glistening, almost colorless, pale grayish-white by reflected light, transparent, somewhat smoky or brownish by transmitted light. Old cultures show the smoky discoloration more distinctly. The growth may also be a conglomeration of minute dew-drop-like colonies. Superficial discrete colonies may somewhat exceed one millimeter in diameter.

*Glycerin Agar.*—Growth more profuse than on plain agar, but much the same.

*Ascitic-fluid Agar.*—Growth more profuse and opaque than on plain agar.

*Glucose Agar Stab.*—Moderately profuse growth along line of inoculation. No gas. No gas in liquefied glucose agar allowed to solidify.

*Broth.*—Turbidity in twenty-four hours. In three to four days this settles forming a whitish sediment with over-lying fluid clear.

*Fermentation Reactions.*—Acidity in glucose and lactose fermentation tubes. No gas. Turbidity in both arms. Indicators decolorized in the closed arms.

*Potato.*—Minute colonies, in about thirty-six hours elevated, moist, somewhat dirty-white with a pasty consistence. Growth in seventy-two hours confluent, later dry and brownish. Growth may fail on potato.

*Gelatin Stab.*—Opaque granular growth along line of inoculation with a slight surface cupping in thirty-six hours. The cupping extends slowly downward producing a tubular

liquefaction which gradually involves the whole medium. Liquefaction slower than with *Micrococcus aureus*.

*Gelatin Plates*.—Small, pale, granular colonies, yellowish by transmitted light, floating in small areas of liquefied gelatin.

*Coagulated Blood-serum*.—Profuse growth like that on agar, but somewhat transparent, medium somewhat translucent and depressed along line of growth with the production of a clear fluid and a breaking down of the solid medium.

*Milk*.—The reaction is characteristic. In neutral litmus milk the litmus is decolorized. Within four hours, a thin blue layer remaining on the surface. On shaking the color returns but is somewhat redder. The reaction is evidently due to deoxidation. In twenty-four hours the milk is firmly coagulated, reddish or bluish on the surface and colorless below. The upper layers of the coagulum become translucent and are gradually changed to a somewhat turbid fluid which is definitely red in the upper portion and yellowish below. The liquefaction of the coagulum is progressive and it is gradually transformed to a flocculent granular material floating in a yellowish liquid. Finally the whole coagulum is transformed and the precipitate stained red throughout. Eventually the milk culture is converted into a clear, straw-colored supernatant fluid, overlying a dark red sediment.

**Production of Enzymes.**—Coagulated milk cultures passed through a Pasteur filter and added in small quantities to sterilized milk produce no appreciable change in reaction, but within thirty-six hours a coagulum is formed quite as firm as that produced by the living organism. The medium is not decolorized or acidified. The organism produces, therefore, a rennin-like ferment. The filtrate from a milk tube in which the coagulum is being dissolved, added to milk produces the typical coagulum which is slowly, but not completely softened and liquefied. This filtrate also produces a liquefaction of sterile nutrient gelatin. The organism thus produces proteolytic enzymes as well as the coagulating enzymes.

No indol.

**Vitality.**—Organisms survive in agar cultures for long periods, four to five months. They are partially destroyed at 62° C. in five minutes and completely destroyed at 65° C. in the same time. They are not destroyed by exposure to 60° C. for five minutes. They are relatively resistant to disinfectants such as carbolic acid and chloroform water.

**Pathogenic Action.**—Pathogenic to white mice by intraperitoneal inoculation in doses of 0.3 to 0.7 c.c. of a thin suspension in broth and by subcutaneous inoculation of larger quantities, 0.8 to 1.8 c.c., the animals dying in seven hours to three to four days of general infection.

Occasionally local abscesses are produced in rabbits by subcutaneous inoculation and general septicemia by intravenous inoculation with vegetations on the mitral valves. In dogs aortic endocarditis may be produced by Rosenbach's procedure of tearing the aortic valves and injecting the organisms into the left jugular vein.

No evidence of soluble toxin.

1. MacCallum and Hastings: J. Exper. M., 1899, iv, 521.
2. Harris and Longcope: Centralbl. f. Bakteriol. [etc.], 1901, xxx, 353.

### GENUS 6. MICROCOCCUS Cohn, emended Winslow and Rogers

Facultative parasites or saprophytes. Cells in plates or irregular masses (never in long chains or packets). *Generally decolorize by Gram.* Growth on agar abundant, with formation of yellow pigment. Glucose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied, but not rapidly.

*Producing yellow pigment*

Non-liquefying

Type species *Micrococcus luteus* (Schröter) Cohn.
" *varians* Migula.

Liquefying

*Micrococcus flavus* (Flügge) Lehmann and Neumann.
" *conglomeratus* (Flügge) Migula.
" *cremoides* Zimmermann.
" *liquefaciens* (Flügge) Migula.

*Producing orange pigment*

Non-liquefying

*Micrococcus aurantiacus* (Schröter) Cohn.

Liquefying

*Micrococcus cremoides aureus* Dyar.

*Not producing pigment*

Non-liquefying

*Micrococcus epidermidis* (Kligler) Hucker.
" *ureæ* Cohn.
" *candidus* Cohn.

Liquefying

*Micrococcus candicans* Flügge.
" *Freudenreichii* Guillebeau.
" *casei* (Jensen) Hucker.

*Producing brownish pigment*

*Micrococcus fuscus* Eisenberg.

*Producing violet pigment*

*Micrococcus violaceus* Cohn.

### MICROCOCCUS LUTEUS (Schröter) Cohn

Originally described as *Bacteridium luteum* by Schröter[1] and as *Micrococcus luteus* by Cohn.[2]

**Morphology.**—Small cells about 0.8 $\mu$ in diameter, generally somewhat ellipsoid in shape as a result of cell division. They may be as much as 1.2 $\mu$ in length.

*Gram-negative.*

**Cultivated** only under aërobic conditions.

*Agar Slant.*—Slimy yellow expansion.

*Gelatin Plates.*—Colonies on the surface 1 to 2 mm. in size, at first whitish-yellow, then citron-yellow, moist, and viscid. They are fairly thick, have irregular edges and are slightly granular under low power. The deep colonies are irregular, round, or whetstone in shape, opaque with smooth sharply defined edges. Old colonies 4 mm. in diameter and 0.5 mm. thick. No liquefaction.

*Gelatin Stab.*—Citron-yellow membranous growth at the surface at first thick, later folded. Coarsely granular colonies in the upper portions of the line of puncture.

*Potato.*—Irregular, thick, moist expansion of a deep sulphur-yellow color. The color deepens with age, the expansion becomes dull and folded.

*Fluid Cultures.*—Thick yellow membranous surface growth, especially in sugar solutions. These become folded and eventually sink to the bottom. Finely granular sediment.

*Pigment* not soluble in water, ether, or chloroform and not changed by acids or alkalies.

Hucker[3] finds that this species does not reduce nitrates or produce sufficient acid to coagulate milk. It can utilize ammonium salts as the only source of nitrogen. Common in milk and milk products.

1. Schröter: Pilze in Kryptog. Flora von Schlesien. 1886, p. 144.
———: Beitr. z. Biol. d. Pflanz., 1872, i, Hft. 2, 119.
2. Cohn: Beitr. z. Biol. d. Pflanz., 1872, i, Hft. 2, 127.
3. Hucker: Studies on the Coccaceæ: IV. The classification of genus micrococcus. Cohn, N. Y. State Agri. Exper. Sta., Tech. Bull. No. 102, June, 1924.

### MICROCOCCUS VARIANS (Dyar) Migula

Isolated originally by Dyar[1] from milk and described as *Merismopedia flava varians.* Named by Migula.[2] According to Hucker this species represents the type of yellow non-liquefying saprophytic micrococcus. Description from Dyar.

**Morphology.**—Micrococci about 1 $\mu$ in diameter in twos, fours, and bunches. Generally Gram-negative (Hucker).

**Growth** aërobic.

*Glycerin Agar.*—Abundant, shining, bright yellow, opaque growth.

*Plain Agar.*—Abundant, yellow growth (Hucker).

*Gelatin Plates.*—Surface colonies much larger than the deep. They are opaque, scarcely shining, light yellow, the edges a little wavy. The deep colonies are round, opaque, yellow, inclined to be slightly irregular. Gelatin not liquefied in sixty days.

*Milk* coagulated in six days or later, but only in boiling. Action on milk variable according to Hucker.

*Broth.*—Fine turbidity and considerable granular yellow sediment.

*Potato.*—Abundant bright yellow growth like that on glycerin agar.

*Dextrose.*—Generally fermented without the formation of gas (Hucker).

*Nitrates* reduced strongly even in seven days.

*Ammonium salts* utilized as the only source of nitrogen (Hucker).

Media with lactose and litmus show a rapid reddening, but in fifty to sixty days a blue color develops in the upper part of the culture.

1. Dyar: Rep. New York Acad. Sc., 1893–1895, viii, 322.
2. Migula: p. 135.

### MICROCOCCUS FLAVUS (Flügge) Lehmann and Neumann

Originally described as *Micrococcus flavus liquefaciens* by Flügge.[1] Named *Micrococcus flavus* by Lehmann and Neumann[2] and by Migula.[3]

**Morphology.**—On plain agar twenty-four hours old the organisms appear as micrococci measuring 0.5 to 0.6 $\mu$ in diameter, in single elements, tetrads, small bunches, and irregular chains. No enlargement on dextrose agar.

**Staining Reactions.**—Stained easily with ordinary anilin dyes, best with gentian-violet. Gram-positive.

**Cultivated** readily on ordinary media. Growth slow at 22° C. and 37° C.

*Plain Agar Plates.*—The organisms grow slowly, colonies appearing in forty-eight hours, pin-point to $\frac{1}{2}$ mm. in size. Under low power they are flat, opaque, granular, edges entire. Deep colonies ovals and disks. In three days the colonies are somewhat increased in size. In five to six days they are 3 to 4 mm. in size, bright yellow or lemon-yellow in color. Bottom colonies colorless. At the end of two weeks the surface colonies are lemon-yellow, elevated, shiny, 7 to 8 mm. in diameter. Under low power they are granular, edges entire. Deep colonies ovals and disks.

*Plain Agar Slant.*—In twenty-four hours a moist, moderately thick, non-spreading lemon-yellow growth. In three to five days it is more abundant, lemon-yellow in color. In two weeks it is very abundant, moist, lemon-yellow and tends to spread over the agar.

*Plain Agar Stab.*—Moderate line and surface growth in twenty-four hours. In three days more abundant surface growth, yellow in color. In two weeks moderate line growth, abundant lemon-yellow surface growth.

*Dextrose Agar Plates.*—No colonies in twenty-four hours. In forty-eight hours small, delicate, pin-point colonies appear. Under low power they are delicate ovals and disks. In five to six days the colonies are yellow, 2 to 3 mm. in size, raised, elevated, and smooth. In seven to ten days the colonies are 3 to 4 mm. in diameter, raised, elevated, smooth, very yellow. On older plates the colonies are raised, umbilicated, and still lemon-yellow in color. No acid.

*Dextrose Agar Slant.*—In twenty-four hours a moist, moderately thick growth turning lemon-yellow. No acid. In three to four days it is very abundant, still lemon-yellow. In two weeks the agar is covered with a moist abundant lemon-yellow expansion. No acid.

*Gelatin Plates.*—Colonies grow slowly, appearing in three days as yellowish dots. Under low power they are round, regular, delicate, granular, opaque, margins entire. In five to six days thickly-seeded plates are liquefied and yellow. Isolated colonies are $\frac{1}{2}$ mm. in size, small, and discrete. After ten to twelve days growth, the colonies are about 1 mm. in size, yellow and hazy, lying in areas of liquid gelatin $1\frac{1}{2}$ cm. in diameter.

*Gelatin Stab.*—Growth slow in line of inoculation and on the surface. Liquefaction appears at the end of about three days on the surface, $\frac{1}{4}$ inch in depth. In six to eight days the tube is half liquefied. Complete liquefaction occurs at the end of ten to twelve days.

*Blood-serum.*—In twenty-four hours a moderate lemon-yellow growth sunk in the serum. In three to five days it is more abundant, yellow, sunken. In two weeks an abundant growth along line of inoculation appears, lemon-yellow in color with considerable softening of the serum. This softening is increased in two to three weeks and eventually the serum is partially liquefied.

*Broth.*—In twenty-four hours turbidity and yellow sediment, no scum. In three to five days increased turbidity and sediment, no scum. In two weeks the turbidity is somewhat lessened, the sediment yellowish, but no scum develops.

*Peptone.*—Turbidity and yellow sediment, no scum.

*Litmus Milk.*—No change in reaction, sediment yellowish. No digestion of the milk proteins.

*Potato.*—In twenty-four hours a moderate lemon-yellow expansion. In three days it is more abundant and quite lemon-yellow. In old cultures the growth is still delicate and lemon-yellow.

*Fermentation Tubes*:

*Dextrose.*—Turbidity and sediment, no scum, arm clear. No acid.

*Saccharose.*—Turbidity and sediment in bowl, no scum, arm clear. No acid.

*Lactose.*—Turbidity and sediment in bulb, no scum, arm clear. No acid.

Hucker reports that this species does not reduce nitrates to nitrites and can grow when ammonium salts are present as the sole source of nitrogen.

1. Flügge: Die Mikroorganismen. 3. Aufl. 1886, ii, p. 174.
2. Lehmann and Neumann: Atlas und Gumdriss der Bakteriologie. 1896, p. 163.
3. Migula: System der Bakterien. 1900, ii, 142.

### MICROCOCCUS CONGLOMERATUS (Flügge) Migula

Originally reported as *Micrococcus citreus conglomeratus* by Flügge.[1] Named by Migula. This type represents the light yellow environmental micrococcus usually called *Micrococcus citreus*. Present description taken from cultures obtained from American Museum.

**Morphology.**—On plain agar twenty-four hours old the organisms appear as small and large cocci arranged singly, in pairs and in irregular bunches. The individual elements are usually spherical and vary in size from 0.4 to 0.7 $\mu$. A few elongated oval forms appear, about 0.8 to 1 $\mu$. in size. On dextrose agar the organisms are about the same size, but larger forms are more common.

**Staining Reactions.**—The organisms stain well with the usual anilin dyes, best with gentian-violet. Gram-positive.

**Cultivated** easily on the usual media. Growth very slow both at 22° C. and at 37° C.

*Plain Agar Plates.*—Colonies grow slowly appearing on the surface in four days, $\frac{1}{2}$ mm. in size, whitish in color. Under low power they are opaque, granular, edges entire, sometimes nucleated. Deep colonies pin-point, under low power ovals and disks. The colonies grow slowly and at the end of ten to twelve days are bright yellow, varying in size from $\frac{1}{2}$ to 5 mm., round and regular, glistening. Under low power they are dark, opaque, finely granular. Bottom colonies thin and colorless, under low power showing very thin peripheries. In eighteen to twenty days the colonies are deep yellow, and measure 5 to 9 mm. in diameter. Bottom colonies colorless, deep colonies oval. Some colonies are almost 1 cm. in size.

*Plain Agar Slant.*—Growth appears slowly, usually only in forty-eight hours, delicate and whitish. In three to four days it is more abundant, slightly yellow. In five to six days it is lemon-yellow in color. In ten to fifteen days it is quite abundant and tends to spread over the agar.

*Plain Agar Stab.*—Delicate line and surface growth in forty-eight hours. In three days it is more abundant along the line and delicate on the surface, light yellow in color. In five to six days it is still more abundant on the surface, and yellow. In ten to fifteen days the surface growth is very yellow, spreads over the agar, with branches extending to the wall of the tube.

*Dextrose Agar Plates.*—Colonies grow slowly appearing in four days, $\frac{1}{2}$ to 1 mm. in size, round, regular, and uniform. Under low power they are round, regular, uniform, yellowish, opaque, edges entire.

Deep colonies pin-point, under low power ovals and disks. No acid. In seven to eight days the surface colonies are yellow, 3 to 4 mm. in size, bottom colonies colorless. In ten to fifteen days the surface colonies are very yellow, raised, smooth and may measure 7 to 8 mm. Deep colonies oval or biconvex disks, $\frac{1}{2}$ mm. in diameter. Bottom colonies colorless. No acid.

*Dextrose Agar Slant.*—In forty-eight hours a moderate, whitish, non-spreading growth. No acid. In three to four days it is more abundant and distinctly yellow. In five to six days it is lemon-yellow in color. After ten to fifteen days the growth is very yellow. No acid.

*Gelatin Plates.*—No growth in twenty-four hours or forty-eight hours. In four days thickly-seeded plates are yellow and filled with masses of fine colonies. Isolated colonies are $\frac{1}{2}$ to 1 mm. in size, yellowish-white. Under low power they are round, regular, uniform, granular, opaque, edges entire. In eight to ten days the colonies are about 1 mm. in size, lying in saucers of liquid gelatin. Later the gelatin is entirely liquefied.

*Gelatin Stab.*—No growth in twenty-four hours. Delicate line and surface growth in forty-eight hours. No liquefaction till growth is five to six days old. At the end of nine to ten days there is a surface liquefaction $\frac{1}{2}$ inch in depth. Completely liquefied in fifteen to twenty days.

*Blood-serum.*—Fairly abundant, yellow, non-spreading growth in forty-eight hours. In three to five days it is more abundant and light yellow. At the end of ten to fifteen days the serum is covered with a yellow expansion. No liquefaction.

*Broth.*—Turbidity and whitish sediment in forty-eight hours. No scum. In three to four days the turbidity and sediment are increased, but no scum develops. In five to six days the turbidity and sediment are much increased. After ten to fifteen days the turbidity is somewhat lessened, a fairly heavy sediment is precipitated but no scum develops.

*Peptone.*—Abundant turbidity and sediment, developing slowly. No scum.

*Litmus Milk.*—Growth slow, the milk clearing slightly in three days. In five to six days it is alkaline and deep blue in color. The milk proteins are slowly dissolved to a muddy yellowish fluid with an alkaline cream ring at the top.

*Potato.*—Growth slow, appearing at the end of five days as a faint yellow expansion. In nine to ten days it is more abundant and lemon-yellow. Potato discolored. In fifteen to eighteen days it is abundant, bright yellow, raised and moist.

*Fermentation Tubes:*

*Dextrose.*—Faint turbidity in bowl in forty-eight hours, arm clear, no scum. No acid.

*Saccharose.*—Faint turbidity in bowl in forty-eight hours, no scum. No acid.

*Lactose.*—Considerable turbidity in bowl in three days, arm clear, no scum. No acid.

Hucker finds that this species does not ferment mannite or glycerin, reduces nitrates to nitrites and can utilize ammonium salts as the sole source of nitrogen.

1. Flügge: p. 182.
2. Migula: p. 146.

### MICROCOCCUS CREMOIDES Zimmerman

Isolated originally from water by Zimmermann. Description from Riley.

**Morphology.**—Small cocci, occurring singly, in pairs, and irregular groups. They measure 0.4 to 0.6 $\mu$ in diameter in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth yellowish-white, at first translucent, later opaque, slightly raised; growth moderate in amount, spreading very little from needle-track except at bottom; margin fairly regular, sharply defined; surface smooth, glistening.

*Agar Stab.*—Filiform growth along needle-track; white slightly translucent growth, spreading in four days over almost whole surface.

*Agar Colonies.*—Colonies white, slightly translucent, later opaque, glistening, round; margins irregular, but sharply defined. Under low power—surface very finely granular; margin sharply defined, smooth except for large, macroscopic irregularities; color gray, darker in center.

*Dextrose Litmus Agar Slant.*—Growth not so large as on plain agar, thinner, and more translucent; medium quickly acidified.

*Dextrose Litmus Agar Plates.*—Colonies round, white, translucent, but sharply defined; plates quickly acidified.

*Gelatin Stab.*—Filiform growth along needle-track, small in amount; no growth, or very small amount, on surface; in one specimen, liquefaction began in ten days and was completed in twenty days; in another, it did not begin until twenty-five or more days; liquefaction saccate, starting in a narrow line along the needle-track, and spreading only gradually to the sides; slight pellicle on surface which soon sinks in the liquid.

*Gelatin Colonies.*—Minute, punctiform; plates liquefied in five to ten days with one specimen, not at all with the other.

*Broth.*—Liquid at first clouded, with a small amount of sediment partly viscid, partly friable; later, liquid clear, sediment increased in amount, with some particles adhering to sides of tube.

*Peptone Solution.*—Growth as in broth.

*Litmus Milk.*—Milk acid in twenty-four hours, coagulated in forty-eight hours; coagulum firm, solid, decolorized; serum clear.

*Potato.*—Rather large amount of white growth, spreading irregularly; rather thin, transparent, and moist.

*Blood-serum.*—Rather large amount of growth, yellowish-white, glistening; medium eventually liquefied throughout.

*Fermentation Tubes:*

*Dextrose Broth.*—Arm clear; bulb heavily clouded with small amount of friable sediment; no pellicle; no gas; reaction strongly acid.

*Lactose Broth.*—Growth as in dextrose broth; reaction strongly acid.

*Saccharose Broth.*—Growth as in dextrose broth; reaction strongly acid.

Zimmermann: Die Bakterien unserer Trink- und Nutzwässer. Chemnitz, 1890, p. 74.

### MICROCOCCUS LIQUEFACIENS (Flügge) Migula

Described originally from ammoniacal urine by Flügge[1] as *Micrococcus ureæ liquefaciens*. Named by Migula.[2]

**Morphology.**—Spherical cells from 1.25 to 2 $\mu$ in diameter, arranged as single elements, in chains of 3 to 10 individuals, or in irregular bunches.

**Cultivation.**—Grows on the usual media at room temperature.

*Gelatin Plates.*—Small white colonies develop in two days appearing under low power as dark gray spherical disks with sharp edges. As the colonies break through to the surface they become much larger, are yellow-brown in color, and show central nuclei. The surface is granular and the edges wavy. Gelatin gradually liquefies.

*Gelatin Stab.*—Whitish confluent growth in the line of stab with a rapid liquefaction of the gelatin which extends to the wall of the tube. The liquid gelatin is turbid and whitish with a thick yellowish-white precipitate at the bottom.

Kligler[3] reports that this species is generally Gram-positive, produces moderate acidity in dextrose, saccharose, and lactose, does not liquefy gelatin, does not reduce nitrates, decomposes peptone to ammonia. It is called *Albococcus urea* (Cohn) Flügge by Kligler.

1. Flügge: Die Mikroorganismen. 3. Aufl. 1896, ii, 173.
2. Migula: p. 106.
3. Kligler: J. Infect. Dis., 1913, xii, 432.

### MICROCOCCUS AURANTIACUS (Schröter) Cohn

First reported as *Bacteridium aurantiacum* by Schröter.[1] Named by Cohn.[2]

**Morphology.**—Round and oval organisms, usually in bunches, rarely as diplococci, about 1.2 $\mu$ in diameter.

**Cultivation.**—*Agar Slant.*—Orange-yellow expansion.

*Gelatin Plates.*—Round and ovoid colonies with smooth sharp edges and glistening surface. Orange-yellow in color. Under the microscope the colonies are finely granular, almost opaque in the middle.

*Gelatin Stab.*—Hemispherical surface growth at point of puncture, orange-yellow. Delicate thread-like growth along line of stab, made up of fine, circular, isolated colonies, growth yellow just beneath the surface, white below. No liquefaction.

*Potato.*—Golden-yellow expansion made up of individual masses, consistency slimy, highly refractile. Similar growth in *cooked egg-albumin.*

*Cohn's Solution.*—Orange-yellow surface layer, several millimeters in height.

Schröter reports the pigment as soluble in water. Schneider[3] and Migula[4] found it insoluble in water. Hucker states that this species generally reduces nitrates and does not utilize ammonium salts when furnished as the sole source of nitrogen.

1. Schröter: Beitr. z. Biol. d. Pflanz., 1872, i, 109.
2. Cohn: Beitr. z. Biol., d. Pflanz., 1872, i, 127.
3. Schneider: Arb. a. d. bakteriol. Inst. d. techn. Hochsch. zu Karlsruhe. 1895.
4. Migula: p. 119.

### MICROCOCCUS CREMOIDES var. AUREUS

Described by Dyar as *Micrococcus cremoides aureus*. Description from Riley.

**Morphology.**—Small cocci, occurring for the most part singly and in pairs, but also in short chains and small groups. They measure 0.5 to 0.7 $\mu$ in diameter in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth white, translucent at first, later opaque; after about five days an orange-yellow tinge appears at the margin, which gradually deepens in color; the amount of orange coloration varies greatly, and at times the whole growth may be orange in color; margin slightly irregular and finely lobed; growth moderate in amount, spreading somewhat at bottom.

*Agar Stab.*—Filiform growth along needle-track; moderate amount of growth on surface, first translucent, later opaque and slightly raised; margin irregular, with orange-yellow tinge; this tinge gradually spreads inward, but not regularly, giving a streaked yellow and white appearance to the surface growth.

*Agar Colonies.*—Round, white, translucent, later opaque and yellowish; margin smooth, sharply defined. Under low power—surface finely granular; margin smooth; color yellowish-gray.

*Dextrose Litmus Agar Slant.*—Growth smaller than on plain agar; color white, but thin and translucent; strong acid formation.

*Dextrose Litmus Agar Plates.*—Colonies smaller than on plain agar; plates acid throughout in two to five days.

*Gelatin Stab.*—Filiform growth along needle-track; moderate amount of growth on surface, white, with yellow tinge at margin, which gradually spreads inward until whole surface growth may become yellowish orange; liquefaction begins generally in six to eight days, but under unfavorable conditions may be delayed for thirty or more days; liquefaction at first saccate, then stratiform, with a thin pellicle which soon sinks.

*Gelatin Colonies.*—Colonies round, with smooth, sharp edge, yellowish in color, surrounded in about ten days by a zone of liquefied gelatin.

*Broth.*—Liquid clouded throughout; moderate amount of viscid sediment; no pellicle observed.

*Peptone Solution.*—Growth as in broth, but smaller in amount.

*Litmus Milk.*—Becomes acid in two to three days, and coagulates slowly, the time of coagulation varying in different specimens from seven to forty days. In one specimen, the coagulum was later digested; in others, partially so.

*Potato.*—Very small amount of growth, so thin and transparent as to be scarcely visible; color yellowish-white.

*Blood-serum.*—Growth at first thin and translucent, later opaque and slightly raised; color white, with orange-yellow margins; no liquefaction, except a very slight amount in one specimen.

*Fermentation Tubes:*

*Dextrose Broth.*—Arm clear; bulb clouded; moderate amount of viscid sediment; no pellicle; no gas formation; reaction acid.

*Lactose Broth.*—Growth as in dextrose broth; reaction acid.

*Saccharose Broth.*—Less growth than in dextrose broth; reaction acid.

Dyar: "On Certain Bacteria from the Air of New York City," in Report of the New York Academy of Science, 1895, viii, p. 349.

### MICROCOCCUS EPIDERMIDIS (Kligler)

Regarded by Kligler as *Albococcus epidermidis* as the type of non-liquefying reducing micrococcus. According to Hucker this species produces a luxuriant white growth on agar slants. It reduces nitrates to nitrites. Does not liquefy gelatin. Generally ferments dextrose, lactose, glycerin, and mannite. Generally does not curdle milk. Gram stain variable. Utilization of ammonium salts as the only source of nitrogen variable.

Kligler: J. Infect. Dis., 1913, xii, 432.

### MICROCOCCUS UREÆ Cohn

First observed by Pasteur[1] in urine which had turned alkaline and regarded by him as a ferment which converts urea into ammonium carbonate. Cohn[2] found spherical or oval cells either single, in chains of 2, 4 and 8 cells, or in irregular masses. Description from Migula.[3]

**Morphology.**—Spherical cocci, arranged as single cells, diplococci and tetrads. Average diameter 0.8 to 1 $\mu$.

**Cultivation.**—*Agar Slant.*—White, mother-of-pearl expansion.

*Gelatin Plates.*—Small, white, somewhat transparent, viscid colonies with sharply defined smooth edges and slight lustre. They are easily lifted from the medium and resemble drops of stearin. The colonies remain small or grow to 10 to 12 mm. in diameter. Old colonies are split and distorted. Under low power the colonies are finely granular on the edges, opaque in the center. Gelatin not liquefied.

*Gelatin Stab.*—Superficial expansion at point of puncture, like colonies on gelatin plates. Thin tenacious thread along the stab. In old cultures an unpleasant odor develops like decomposed paste.

Urea converted to ammonium carbonate.

According to Hucker this species does not reduce nitrate and does not ferment mannite. It can utilize urea and ammonium salts as an only source of nitrogen. Gram stain variable.

1. Pasteur: Ann. de chim. et phys., 1862, lxiv, 52; 55.
2. Cohn: p. 158.
3. Migula: p. 62.

### MICROCOCCUS CANDIDUS Cohn

Mentioned originally by Cohn[1] as the cause of white flecks and small masses on cooked potatoes. Description from Migula.[2] Common in air and water.

**Morphology.**—Small spherical cells about 0.5 to 0.7 $\mu$ in diameter forming on solid media white viscid hemispherical drops which quickly dry down to chalk-white crusts. The viscid material dissolves rapidly in water.

**Cultivation.**—*Agar Slant.*—Pure white, elevated, narrow growth with edges slightly folded after several days.

*Gelatin Plates.*—Snow-white, slightly raised colonies whose edges quickly become irregular. Under low power the colonies are definitely granular. No liquefaction.

*Gelatin Stab.*—Snow-white growth on the surface, button-like, raised, with smooth edges, later becoming flat with irregular edges.

Hucker has reported that this species does not reduce nitrates, generally ferments dextrose, lactose, glycerin, and mannite. Usually does not curdle milk. Different strains vary in their ability to utilize ammonium salts as the only source of nitrogen. Gram's stain variable.

A species identified as *Micrococcus candidus* was found several times in war wounds by Weinberg and Séguin.[3]

1. Cohn: Beitr. z. Biol. d. Pflanz. 1872, i, 127.
2. Migula: p. 47.
3. Weinberg and Séguin: Le gangrène gazeuse.

### MICROCOCCUS CANDICANS Flügge

Isolated originally by Flügge. Present description from Riley's observations on air organisms in Baltimore.

**Morphology.**—Medium-sized cocci, varying greatly in size, occurring singly, in pairs, short chains, and irregular groups. They measure 0.5 to 0.7 $\mu$ in diameter in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth white, at first translucent, later opaque and slightly raised; surface smooth, glistening; margin thinner, more translucent; edge irregular, finely lobed; growth moderate in amount, spreading a little at bottom.

*Agar Stab.*—Filiform growth along needle-track; moderate amount of growth on surface, white, with irregular edge.

*Agar Colonies.*—Round, white, glistening; margin slightly irregular. Under low power—margin smooth, sharply defined; surface finely granular; color dark gray.

*Dextrose Litmus Agar Slant.*—Growth somewhat smaller than on plain agar; color white, slightly translucent; strong acid formation.

*Dextrose Litmus Agar Plates.*—Colonies round, white, slightly translucent; plates acid in two to five days.

*Gelatin Stab.*—Filiform growth along needle-track; growth on surface white, very irregular, lobed; no liquefaction for three months, when a small amount of saccate liquefaction developed along the line of the stab.

*Gelatin Colonies.*—Round, white, opaque; plate partly liquefied in five to ten days.

*Broth.*—Liquid evenly clouded; small amount of viscid sediment; no pellicle.

*Peptone Solution.*—Liquid faintly clouded; small amount of sediment; no pellicle.

*Litmus Milk.*—Milk acid in fifteen days, but no coagulation.

*Potato.*—Small amount of growth, white, opaque, raised.

*Blood-serum.*—Rather large amount of growth, white, glistening; no liquefaction.

*Fermentation Tubes:*

*Dextrose Broth.*—Arm clear; bulb faintly clouded; small amount of sediment; no pellicle; no gas formation; reaction slightly acid.

*Lactose Broth.*—Growth as in dextrose broth; reaction neutral.

*Saccharose Broth.*—Less growth than in dextrose broth; reaction neutral.

Flügge: Die Mikroorganismen. 2. Aufl. 1886, ii, 173.

### MICROCOCCUS FREUDENREICHII Guillebeau

Originally obtained from milk by Guillebeau.[1] According to Hucker this species is a facultative parasite which represents the non-pigmented liquefying micrococci. Description from Bergey[2] and Hucker.

**Morphology.**—Micrococci 0.6 to 1.2 $\mu$ in diameter occurring singly, rarely in short chains. Also in clumps (Hucker).

Gram-positive (Bergey). Gram variable (Hucker).

**Cultivation.**—*Growth* aërobic, best at 20° C.

*Agar colonies* white, slimy.

*Agar slant* white, smooth.

*Gelatin colonies* small, white, and opaque.

*Gelatin Stab.*—Infundibuliform liquefaction.

*Broth.*—Turbidity with white sediment.

*Litmus Milk.*—Acid coagulated and peptonized according to Bergey. Hucker states that this species does not produce sufficient acid to curdle milk.

*Potato.*—Moderate white to yellow streak.

Acid in dextrose, saccharose, and lactose. Generally does not ferment mannite or glycerin (Hucker).

Nitrates not reduced to nitrites.

No indol.

Ammonium salts utilized as the sole source of nitrogen.

1. Guillebeau: Landwirtsch. Jahrb. D. Schweitz., 1892, Bd., 5, p. 135.
2. Bergey: p. 66.

### MICROCOCCUS CASEI (Jensen) Hucker

Reported primarily as *Micrococcus casei liquefaciens* by Jensen from Emmentaler cheese. According to Hucker this species may be regarded as the type of saprophytic liquefying white micrococci.

Forms large clumps of cells. Usually Gram-positive, but reaction to Gram's stain may vary with different strains. Luxuriant white growth on agar slants. Gelatin liquefied. Acid clot in milk which is rapidly peptonized. Ammonium salts utilized as the only source of nitrogen. Dextrose and lactose fermented with acid but no gas.

According to Jensen *Micrococcus casei* can produce from peptone not only valerianic acid and butyric acid, but also acetic and formic acids.

Jensen: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1904, xiii, 161.

### MICROCOCCUS FUSCUS Eisenberg

A micrococcus producing a brown pigment was originally cultivated by Maschek[1] from drinking water and named by Eisenberg.[2] According to Maschek the organisms were very irregular in shape, either cocci or short bacilli, produced pale brown to black colonies on gelatin which was rapidly liquefied. The gelatin cultures had a strong putrefactive odor. On potato the growth was viscid, at first brown, later almost black.

1. Maschek: Jahresb. d. Kommunal—Oberrealschule zu Leitmeritz, 1887, No. 6, p. 60.
2. Eisenberg: Bakteriologisce Diagnostik. 3. Aufl. 1891, p. 6.

### MICROCOCCUS VIOLACEUS Cohn

Described by Maschek[1] from drinking water. Named by Cohn.[2] Description from Migula.[3]

**Morphology.**—Small spherical cells often arranged in short chains.

**Cultivation.**—*Agar Slant.*—Slow growth with a violet expansion covering the surface.

*Gelatin Plates.*—Hemispherical viscid button-like colonies raised from the surface of the gelatin. These viscid colonies spread slowly, but do not liquefy the gelatin. Under low power colonies three days old look like half spheres.

*Gelatin Stab.*—Slight growth along line of stab with some violet pigment found, especially just beneath the surface. Rather abundant button-like growth at point of puncture.

*Gelatin Slants.*—Abundant pigment production.

*Potato.*—Slow growth. Cultures four weeks old show an intense dark violet color.

*Cooked Rice.*—Violet color not so marked as on potato.

1. Maschek: Jahresb. d. Kommunal-Oberrealschule zu Leitmeritz., 1887, No. 21, p. 67.
2. Cohn: Beitr. z. Biol. d. Pflanz., 1872, i, 155.
3. Migula: p. 186.

### GENUS 7. SARCINA Goodsir, emended Winslow and Rogers

SARCINA differs from MICROCOCCUS solely in the fact that cell division occurs under favorable conditions in three planes, forming regular packets.

*Pigmented*

Type species *Sarcina ventriculi* Goodsir.
" *lutea* Schröter.
" *flava* De Bary.
" *subflava* Ravanel.
" *aurantiaca* Flügge.
" *flavescens* Henrici.
" *carnea* Gruber.
" *rosea* Lindner.

*Non-pigmented*

*Sarcina minuta* De Bary.
" *alba* Zimmermann.
" *candida* Reinke.
" *pulmonum* Virchow.

#### SARCINA VENTRICULI Goodsir

Described first by Goodsir from the contents of a dilated stomach. Individual organisms transparent, pale yellow or brown. Cell walls rigid. Whole organisms had the appearance of a wool pack, or of a soft bundle bound with cord crossing it 4 times at right angles and at equal distances. Individual organisms varied from $\frac{1}{800}$ to $\frac{1}{1000}$ of an inch in linear dimensions measured along their straight sides. Stained deep brown or yellow with iodin. Shriveled slightly in alcohol and nitric acid. Each organism consisted of 16 4-celled frustules. Fission of each cell into 4, giving organisms of 16 cells and multiples of 16 cells.

A strain of *Sarcina ventriculi* obtained from the American Museum had the following characteristics:

**Morphology.**—Large cocci 0.8 to 0.9 $\mu$ in diameter in plain agar cultures twenty-four hours old. No change in size on dextrose agar.

**Staining Reactions.**—Gram-positive.

**Cultivation.**—*Plain Agar Plates.*—Grow slow, appearing only in four or five days. Colonies 3 to 4 mm. in size, round, regular, elevated. Under low power very opaque, almost black, granular, margins entire. Deep colonies ovals and disks. Under low power very dark, wedge-shaped and oval. Very faint yellow color in surface colonies. At the end of two weeks colonies are slightly yellow, measuring 6 to 8 mm. on the surface; bottom colonies colorless; deep colonies ovals and disks. Under low power colonies still have entire margins and are dark and granular.

*Plain Agar Slant.*—In twenty-four hours, thick, non-spreading, slightly yellowish growth. In three days more abundant, slightly yellow. In two weeks abundant, moist, slightly yellow.

*Plain Agar Stab.*—In twenty-four hours moderate line and surface growth. In three days more abundant growth, yellow on the surface. In two weeks very abundant cream-yellow surface growth, moderate line growth.

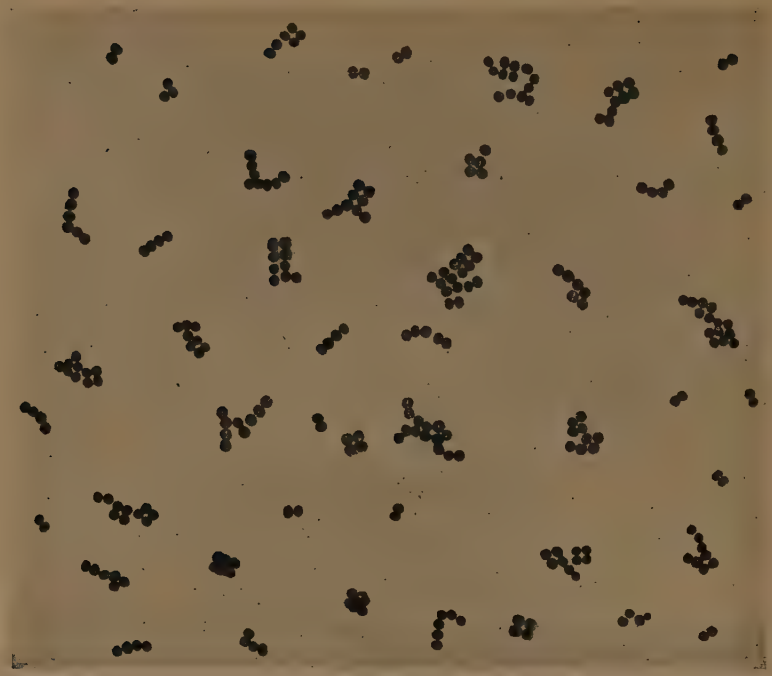

Fig. 64.—*Sarcina ventriculi* from twenty-four-hour culture on plain agar, stained with gentian-violet.

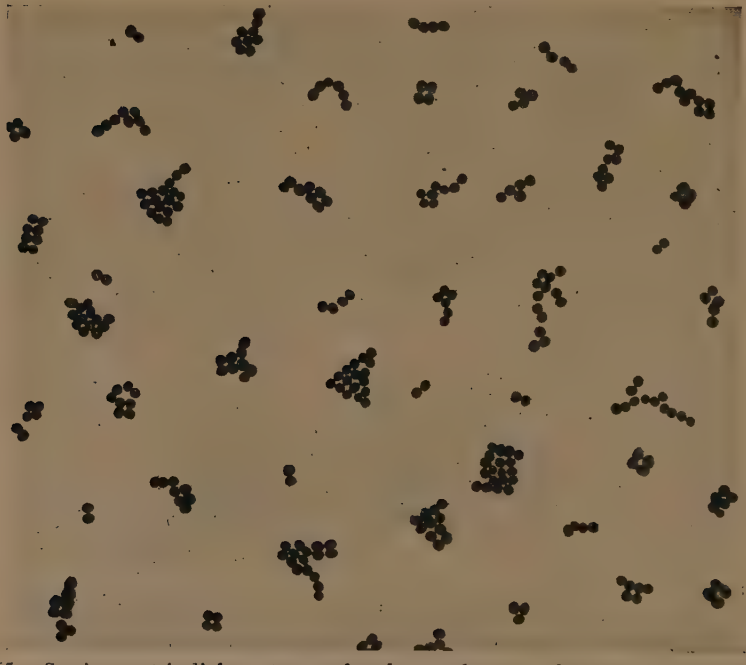

Fig. 65.—*Sarcina ventriculi* from twenty-four-hour culture on dextrose agar, stained with gentian-violet.

*Dextrose Agar Plates.*—Colonies appear in forty-eight hours, ½ mm. in size on the surface, small and delicate. Under low power dark, opaque, granular, edges entire. Deep colonies pin-point. Under low power ovals and disks, dark, opaque and granular. In five days colonies quite yellow, raised, elevated, 2 to 3 mm. In seven days colonies 3 to 4 mm., very yellow, raised, elevated. In eighteen days colonies 4 to 5 mm., pale yellow, umbilicated, nucleated. Deep colonies ½ mm., round and oval; bottom colonies colorless. Colonies have a yellowish color which is different from that of *Micrococcus citreus*, *Sarcina lutea* or *Sarcina flava*.

*Dextrose Agar Slant.*—In twenty-four hours thick, non-spreading, slightly yellow growth, no acid. In three days more abundant, moist, lemon yellow. In two weeks abundant, moist, slightly yellow growth, alkaline reaction.

*Broth.*—In twenty-four hours turbidity, filmy sediment, no scum. In three days turbidity, sediment and filmy scum. In two weeks heavy sediment, considerable filmy scum; broth clear, rather thick and viscid.

*Peptone.*—In twenty-four hours turbidity, filmy sediment, no scum. In three days turbidity, sediment, filmy material in upper part of the tube. In two weeks some sediment and filmy scum; broth clear.

*Litmus Milk.*—In twenty-four hours no change. In three days no change. In two weeks no change.

*Potato.*—In twenty-four hours faint yellow growth. In three days, dry, slightly yellow. In two weeks raised, dry, corrugated growth, rather yellow.

*Blood-serum.*—In twenty-four hours thick, slightly yellow, non-spreading growth. In three days more abundant, slightly yellow. In two weeks abundant, slightly yellow growth, no liquefaction, but considerable softening of the gelatin. No definite liquefaction in twenty-seven days.

*Gelatin Stab.*—In three days delicate line and surface growth. No liquefaction. In six days beginning surface liquefaction. Liquefaction complete in eleven to fifteen days.

*Gelatin Plates.*—Colonies appear in three days, small and delicate. Under low power dark, opaque, granular, with irregular fuzzy outgrowths, margins irregular. In five days colonies slightly yellow, ½ mm. in size. In seven days isolated colonies are about 1 mm. in diameter, slightly yellow, lying in cups of liquid gelatin. In nine days the gelatin is liquefied, colonies ½ to 1½ mm., slightly yellowish, hazy looking. In thirteen days all the plates completely liquefied and have a slightly yellowish tinge.

*Fermentation Tubes:*

*Dextrose.*—In twenty-four hours turbidity, filmy sediment, no scum, arm clear. In three days the same, arm clear, no acid.

*Saccharose.*—In twenty-four hours turbidity, filmy sediment, no scum, arm clear. In three days the same, arm clear. No acid.

*Lactose.*—In twenty-four hours turbidity, filmy sediment, no scum, arm clear. In three days the same, arm clear, no acid.

Goodsir: Edin. M. and S. J., 1842, lvii, 432.

### SARCINA LUTEA Schröter

Isolated by Schröter.[1] Present description from Riley.[2]

**Morphology.**—Rather small sarcinæ, varying in size, occurring for the most part in regular packets, singly, or in pairs, but sometimes in irregular groups. They measure 0.6 to 1.2 $\mu$ in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth lemon yellow, at first translucent and rather dull, later opaque and bright in color, rather thick, raised; surface smooth, glistening; margin somewhat irregular, at times finely lacerated; growth moderate in amount, spreading very little from needle-track, except at bottom.

*Agar Stab.*—Filiform growth along needle-track; small amount of growth on surface, raised, opaque, yellow, spreading irregularly.

*Agar Colonies.*—Round, with smooth edges, lemon-yellow, opaque; surface smooth, glistening. Under low power—margin smooth, sharply defined; surface finely granular; color yellowish-gray.

*Dextrose Litmus Agar Slant.*—Growth slightly stronger than on plain agar; color paler, often appearing whitish; medium acid at first, but later often turned alkaline, though only in the case of very strong growth.

*Dextrose Litmus Agar Plates.*—Colonies round, whitish, opaque; plates acid, sometimes turning alkaline in regions of densest growth.

*Gelatin Stab.*—Filiform growth along needle-track; small amount of growth on surface, raised, spreading, slightly yellow, opaque; liquefaction very slow, beginning in twenty to forty-five days and proceeding slowly; liquefaction saccate at first, later stratiform; liquefied portion clouded, with a yellow sediment.

*Gelatin Colonies.*—Small, round, yellowish, raised; colonies surrounded by a liquefied zone after about a month.

*Broth.*—Liquid clouded at first, with small amount of viscid sediment; later, liquid clear, with large amount of sediment, very viscid and stringy, yellow; no pellicle.

*Peptone Solution.*—Less growth than in broth.

*Litmus Milk.*—Unchanged.

*Potato.*—Growth small in amount and often lacking; raised, bright yellow, rather dry; surface granular.

*Blood-sreum.*—Large amount of growth, spreading finally over entire surface, lemon yellow; surface smooth, glistening, moist; moderate amount of liquefaction, beginning only after twenty or more days.

*Fermentation Tubes:*

*Dextrose Broth.*—Arm clear; bulb clouded, with a small amount of sediment; no gas formation; no pellicle; reaction acid in two to three days, neutral or alkaline in four.

*Lactose Broth.*—Less growth than in dextrose broth; reaction neutral.

*Saccharose Broth.*—Less growth than in dextrose broth; reaction neutral.

**Remarks.**—The liquefaction of gelatin by *Sarcina lutea* was not found by Schröter,[1] but is spoken of by Migula,[3] and Chester.[4] Chester described *Sarcina lutea* as coagulating milk, a character which was not shown by this organism, and which is not mentioned by any of the other authorities.

1. Schröter: Kryptogamenflora von Schlesien. 1886, iii, p. 154.
2. Riley: Air Organisms in Baltimore.
3. Migula: 1900, ii, 247.
4. Chester: A Manual of Determinative Bacteriology. New York, 1914.

### SARCINA FLAVA De Bary

Described by De Bary.[1] Organisms identified as this species have been found by many authors in air, water, dust, in beer, etc. Description from Migula.[2]

**Morphology.**—Spherical cells arranged in packets of 16 to 32 elements combined to form groups of varying sizes. Average size of individual cells 1 to 2 $\mu$ according to DeBary, 0.8 to 2.5 $\mu$ according to Lindner.[3]

**Cultivation.**—Growth fairly rapid at room and body temperatures. Strongly aërobic.

*Slant Agar.*—Yellowish expansion.

*Gelatin Plates.*—Slow growing, small, round, yellowish colonies which liquefy somewhat on the fourth day. By the seventh day a zone of liquefaction is formed about the colonies.

*Broth* containing *peptone.* Abundant growth. Sediment at first white, then yellowish. Turbidity in old cultures.

*Hay Infusion.*—Slow formation of a surface membrane in which packets of pigmented and non-pigmented sarcinas are present.

*Potato.*—Yellow expansion, limited to line of inoculation.

The yellowish pigment turns dirty-green on the addition of $H_2SO_4$, the yellow color being restored by NaOH.

1. De Bary: Vorlesungen über Bakterien, 1887, No. 3, p. 151.
2. Migula: p. 232.
3. Lindner: Die Sarcina-Organismen der Gärungsgewebe. Diss. Berlin, 1888, p. 50.

### SARCINA SUBFLAVA Ravanel

Found by Ravanel in the surface soil from a cultivated field.

**Morphology.**—Large packets of cocci, with 4, 8, 16, 32, or more elements on each face.

**Cultivated** only under aërobic conditions, both at 22° C. and at 35° to 36° C.

*Colonies on Gelatin Plates.*—Colonies become visible in thirty-six to forty hours as minute yellowish dots. With a magnification of 80 diameters they are finely granular and yellowish disks with even margins. By the fourth day the surface colonies are 1 mm. in diameter, pale yellow, and slightly sunken in the gelatin. Under low power they are pale yellow, homogeneous, and have even margins. After some days the margins may become irregular and look more or less granular. The deep colonies increase very slowly and only change in appearance by becoming more dense. Liquefaction is rather slow. There is nothing at all distinctive about the colonies.

*Agar Slant.*—A yellowish band, 3 mm. wide, forms by the fourth or fifth day. It is smooth, pale yellow, and has irregular beaded margins.

*Gelatin Stab.*—Growth quite deep along the puncture with a small almost white button on the surface, which has irregular, beaded margins. Slow liquefaction takes place under this in saucer shape. At the end of ten days the liquefaction is 1 cm. deep and has a level floor. The liquid is cloudy.

*Potato.*—No growth.

*Bouillon.*—Whitish flocculi at the bottom and after several days a thin pellicle on the surface. No cloudiness.

*Rosolic Acid.*—No change.

*Litmus Milk.*—No change either in color or in the milk itself after four weeks. Reaction faintly acid.

*Sugar Gelatin Deep Stab.*—Growth quite deep and on the surface with slow liquefaction. No gas production.

Indol not formed.

Ravanel: Mem. Nat. Acad. Sc., 1896, viii, 10.

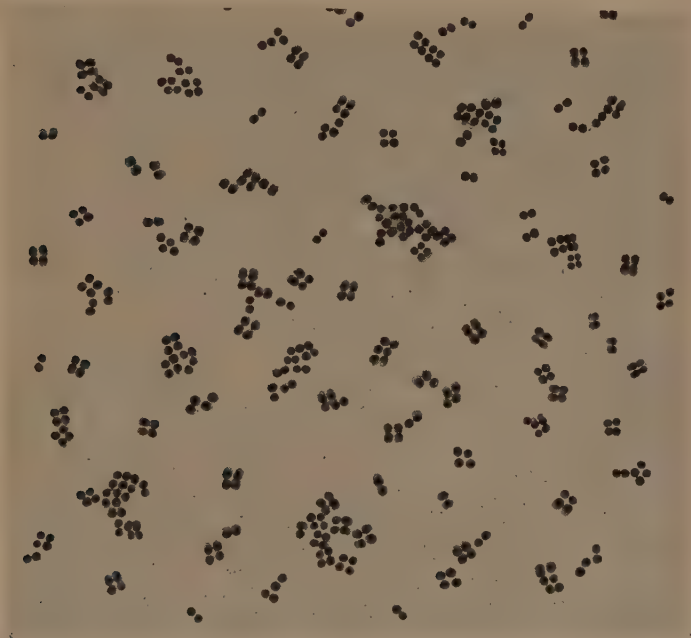

Fig. 66.—*Sarcina subflava* from plain agar twenty-four hours old, stained by Gram.

### SARCINA AURANTIACA Flügge

Described originally by Flügge.[1] Description from Riley.[2]

**Morphology.**—Medium-sized sarcinæ, not very regular, occurring in typical packets, but also singly, in pairs, and irregular groups. They measure 0.6 to 0.9 $\mu$ in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth thin, translucent, yellowish-orange, at first pale and scarcely pigmented, later deeper, occasionally opaque and bright orange at bottom; margin at first smooth, becoming later somewhat irregular.

*Agar Stab.*—Filiform growth along needle-track; small amount of pale orange growth on surface.

*Agar Colonies.*—Small, round, pale orange. Under low power—margin smooth, sharply defined; surface finely granular; color yellowish-gray.

*Dextrose Litmus Agar Slant.*—Very small amount of growth, so thin and transparent as to be scarcely visible; color yellowish; medium faintly acid.

*Dextrose Litmus Agar Plates.*—Colonies minute, punctiform; plates faintly acid.

*Gelatin Stab.*—Filiform growth along needle-track, small amount of pale orange growth on surface; liquefaction beginning about the sixth day, but proceeding very slowly, not complete for two or three months; liquefied portion heavily clouded, with a heavy orange sediment.

*Gelatin Colonies.*—Small, round, orange; surface smooth, glistening; liquefaction very slow.

*Broth.*—Liquid slightly clouded at first, but clear in about ten days; moderate amount of viscid sediment, yellowish at first, later orange; no pellicle.

*Peptone.*—Liquid clear, moderate amount of sediment; no pellicle.

*Litmus Milk.*—Unchanged.

*Potato.*—No growth.

*Blood-serum.*—Moderate amount of growth, rather thin, dark orange, glistening; small amount of liquefaction beginning in about thirty days.

*Fermentation Tubes:*

*Dextrose Broth.*—No growth visible; reaction neutral.

*Lactose Broth.*—No growth visible; reaction neutral.

*Saccharose Broth.*—No growth visible; reaction neutral.

**Remarks.**—In Chester,[3] *Sarcina aurantiaca* is described as coagulating and peptonizing milk, but Dyar[4] says that it does not coagulate milk; Migula[5] and Flügge do not mention the reaction in milk. A growth on potato is described in Chester and in Migula.

Kligler[6] states that *Sarcina aurantiaca* is valuable to Gram stain, produces moderate acid in dextrose but not in saccharose and lactose, does not reduce nitrates and decomposes peptones.

1. Flügge: Die Mikroorganismen. Leipzig, 1886, ii, p. 182.
2. Riley: Air Organisms in Baltimore.
3. Chester: A Manual of Determinative Bacteriology. 1914, p. 113.
4. Dyar: On Certain Bacteria from the Air of New York City. Report New York Acad. of Sc. 1895, viii, 353.
5. Migula: System der Bakterien. Jena, 1900, ii, 238.
6. Kligler: J. Infect. Dis., 1913, xii, 432.

### SARCINA FLAVESCENS Henrici

Described by Henrici.[1] Present notes from Riley.[2]

**Morphology.**—Medium-sized sarcinæ, varying in size, occurring for the most part in regular packets, singly, and in pairs. They measure 0.7 to 1.2 $\mu$ in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth pale, sulphur yellow, at first translucent, later raised and opaque; growth moderate in amount, spreading very little beyond needle-track; margin very slightly irregular; surface smooth.

*Agar Stab.*—Filiform growth along needle-track; growth on surface yellowish, thin and translucent, spreading irregularly.

*Agar Colonies.*—Round, yellowish, opaque, margins smooth. Under low power—margin sharply defined, but slightly irregular; surface coarsely granular; color dark gray.

*Dextrose Litmus Agar Slant.*—Growth stronger than on plain agar, yellowish-white, opaque; spreading irregularly at bottom; margin irregular, finely lobed; medium acid at first, but alkalinity developed in twelve to fifteen days.

*Dextrose Litmus Agar Plates.*—Colonies round, yellowish white, opaque; plates acid at first, later alkaline in regions of densest growth.

*Gelatin Stab.*—Filiform growth along needle-track; moderate amount of growth on surface, opaque, somewhat deeper yellow than on agar; liquefaction beginning in twelve to thirty days, crateriform at first, then stratiform; liquefied portion heavily clouded, with yellow sediment at bottom.

*Gelatin Colonies.*—Round, opaque, deeper yellow than on agar; colonies surrounded by a liquefied zone in ten to twelve days.

*Broth.*—Liquid slightly clouded throughout, with viscid, stringy sediment; no pellicle.

*Peptone Solution.*—Less growth than in broth.

*Litmus Milk.*—Unchanged.

*Potato.*—Growth small in amount, thick, raised, dry, rather dull, light yellow.

*Blood-serum.*—Large amount of growth, spreading gradually over whole surface, slightly raised, opaque, yellowish-white; surface smooth, glistening; no liquefaction.

*Fermentation Tubes:*

*Dextrose Broth.*—Arm clear; bulb clouded; moderate amount of sediment, partly viscid, partly friable, adhering to sides of bulb; no gas formation; no pellicle; reaction acid.

*Lactose Broth.*—Arm clear, bulb clouded, with sediment as in dextrose broth; reaction neutral.

*Saccharose Broth.*—Arm clear, bulb clouded, with sediment as in dextrose broth; reaction acid.

**Remarks.**—This organism corresponds with the *Sarcina flavescens* described by Henrici and Migula[3] except that the growth of the latter in broth is said to be clear, whereas this organism clouded the broth faintly.

1. Henrici: Beitrag zur Bakterienflora des Käses. Arbeiten aus dem bakter., Inst. der Tech. Hochschule, 1894, i, 1, p. 91.
2. Riley: Air Organisms in Baltimore.
3. Migula: System der Bakterien. Jena, 1900, p. 237.

### SARCINA CARNEA Gruber

Reported first by Gruber.[1] Present description from Riley.[2] Found by Tissier[3] in the stools of nurslings.

**Morphology.**—Rather small sarcinæ, not very regular, occurring in typical packets, but also singly, in pairs, and irregular groups. They measure 0.6 to 0.9 $\mu$ in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-negative.

**Cultural Characters.**—*Agar Slant.*—Growth pinkish-orange to salmon-pink, pale at first, deeper after five to ten days; pigment not well developed at incubator temperature; growth slightly raised, opaque, spreading very little from needle-track; margin smooth; surface glistening.

*Agar Stab.*—Filiform growth along needle-track; small amount of growth on surface, yellowish, translucent.

*Agar Colonies.*—Round, glistening, yellowish. Under low power—margin smooth, sharply defined; surface rather coarsely granular; color yellowish-gray.

*Dextrose Litmus Agar Slant.*—Small amount of growth, very thin and transparent; color paler than on plain agar; medium acid.

*Dextrose Litmus Agar Plates.*—Colonies small, round, thin, and translucent; plates strongly acid.

*Gelatin Stab.*—Filiform growth along needle-track; small amount of pinkish-orange growth on surface. Only a small amount of saccate liquefaction at the end of three months.

*Gelatin Colonies.*—Minute, punctiform; no liquefaction.

*Broth.*—Liquid clouded at first, later clear; moderate amount of viscid sediment, yellowish at first, later salmon-pink; no pellicle.

*Peptone Solution.*—Growth smaller than in broth.

*Litmus Milk.*—Unchanged.

*Potato.*—No growth.

*Blood-serum.*—Moderate amount of growth, pale pinkish-orange, opaque, slightly raised; surface smooth, glistening, moist; no liquefaction.

*Fermentation Tubes:*

*Dextrose Broth.*—Arm clear; bulb heavily clouded; large amount of sediment, partly viscid, partly friable, no pellicle, but white ring around sides at surface of liquid; no gas formation; reaction acid.

*Lactose Broth.*—Both arm and bulb clear; small amount of friable sediment; no pellicle; reaction neutral.

*Saccharose Broth.*—Both arm and bulb clear; small amount of friable sediment; no pellicle; reaction neutral.

**Remarks.**—*Sarcina carnea* is described as not liquefying gelatin, but only after eight weeks; this organism did not liquefy gelatin in eight weeks, but did after three months.

1. Gruber: Die Arten der Gattung Sarcina. Arbeiten aus dem bakter., Inst. der Tech., Hochschule zur Karlsruhe, 1895, i, 278.
2. Riley: Air Organisms in Baltimore.
3. Tissier, H.: Recherches sur la Flore Intestinale des Nourrissons (Etat normal et pathologique). Paris, 1900, p. 63.

### SARCINA ROSEA Lindner

Described as *Sarcina rosea* Schröter by Lindner, and as *Sarcina rosacea* (Lindner) by Migula. Description from Lindner.

**Morphology.**—Small spherical cells arranged in packets about 0.8 to 1.5 $\mu$ in size. Grows well on artificial media. Produced a reddish expansion on agar, gelatin, malt-extract gelatin, and a reddish sediment in broth. The gelatin is not liquefied. Excellent growth in neutral

malt-extract solution. The red pigment of this organism is soluble in hot alcohol, insoluble in water, chloroform, carbon bisulphid, petrolether, and benzol. It turns blue-green with concentrated $H_2SO_4$, the color being restored by NaOH. The color is not affected by concentrated HCl, NaOH, $NH_4OH$, but disappears on the addition of acetic acid.

Linder: Die Sarcina-Organismen der Gärungsgewerbe. Inaug. Diss., Berlin, 1888, p. 45.

### SARCINA MINUTA De Bary

Originally described by De Bary.[1] Present description from Tissier[2] who found the organism in the stools of nurslings.

**Morphology.**—The stools show tetrads, fluid cultures large diplococci, tetrads, and packets. On solid media the packets are rare, the diplococci more frequent.

**Staining Reactions.**—Stains well with diluted fuschin or gentian-violet. Gram-positive.

**Cultivation.**—Vitality excellent. The cultures remain alive for fifteen days to three weeks. Growth rapid under aërobic and anaërobic conditions at the ordinary temperature and at 37° C.

*Agar colonies* in four days. The colonies are small fine points, as large as the point of a needle. White in color, opaque, circular.

Water of condensation not turbid. Fine granules appear on the edge of the tube.

*Sugar Agar.*—Growth more rapid.

*Gelatin Streak.*—Slow growth, whitish colonies, like those on ordinary agar.

*Gelatin Stab.*—Slow growth of very fine white colonies.

*Potato.*—Very small white elevated colonies in five days.

*Broth.*—No turbidity. Deposit of granules at the bottom of the tube.

*Milk.*—No marked change. No coagulation. Slight acidity in six days.

*Deep Sugar Agar.*—Rapid growth throughout. Fine, regular lenticular colonies. No gas. Non-pathogenic to white mice.

1. De Bary: Vorlesungen über Bakterien. 1887, p. 151.
2. Tissier: Recherches sur la Flore Intestinale des Nourrissons (Etat normal et pathologique). Paris, 1900, p. 61.

### SARCINA ALBA Zimmermann

Found and named by Zimmermann.[1] Description from Riley.[2]

**Morphology.**—Medium-sized sarcinæ, occurring in fresh cultures, for the most part, in regular packets, but in old cultures, very irregularly. They measure 0.75 to 0.9 $\mu$ in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth white, opaque, rather moist; surface smooth, glistening; margin regular; growth moderate in amount, spreading slightly at bottom.

*Agar Stab.*—Filiform growth along needle-track; small amount of growth on surface, white, slightly translucent, somewhat irregular.

*Agar Colonies.*—Round, white, opaque, convex, smooth. Under low power—margin sharply defined, slightly repand; surface very finely granular; color dark gray.

*Dextrose Litmus Agar Slant.*—Growth white, opaque, raised; surface smooth, glistening; margin regular; no acid formation.

*Dextrose Litmus Agar Plates.*—Colonies round, white, convex, no acid formation; after ten to fifteen days a slight alkalinity.

*Gelatin Stab.*—Filiform growth along needle-track; small amount of white, opaque, regular growth on surface, which gradually sinks as the gelatin becomes cupped with slow liquefaction; the actual crateriform liquefaction does not begin for about ten days.

*Gelatin Colonies.*—Minute, punctiform, yellowish-white; cupping of the gelatin takes place around each colony.

*Broth.*—Liquid clouded; small amount of very viscid stringy, sediment; no pellicle.

*Peptone.*—Liquid faintly clouded, later clear; small amount of viscid sediment; no pellicle.

*Litmus Milk.*—Unchanged.

*Potato.*—No growth visible.

*Blood-serum.*—Moderate amount of growth, white, opaque, regular; no liquefaction.

*Fermentation Tubes:*

*Dextrose Broth.*—Arm clear; bulb clouded; small amount of viscid sediment; no pellicle; no gas formation; reaction faintly acid or neutral.

*Lactose Broth.*—Arm clear; bulb clouded; small amount of sediment, reaction neutral.

*Saccharose Broth.*—Arm clear; bulb clouded; small amount of sediment, reaction neutral.

1. Zimmermann: Die Bakterien unserer Trink-und Nutzwässer. Chemnitz, 1890, p. 90.
2. Riley: Air Organisms in Baltimore.

### SARCINA CANDIDA Reinke

First found by Reinke in the air about breweries and described by Lindner.[1] Present description from Tissier,[2] who found the organism in the stools of nursing infants.

**Morphology.**—Large diplococci or tetrads in the stools and also in cultures. In the latter, characteristic packets.

**Staining Reactions.**—Stained by the usual dyes. Gram-positive.

**Destroyed** at 60° C. in fifteen minutes. Organisms die out spontaneously in eight to ten days. Growth under aërobic and anaërobic conditions at room temperature and at 37° C.

**Cultivation.**—*Agar Plates.*—Colonies round, thick, moist, white, adherent to the agar. Under the microscope they have a uniform thickness and clear-cut edges. The colonies may coalesce.

On *agar slants* the water of condensation does not become turbid but a deposit of large granules forms in two to four days.

*Beef Serum.*—Colonies small, moist, white.

*Gelatin Streak.*—Small liquefying white colonies.

*Gelatin Stab.*—Liquefaction begins in twenty-four hours, complete in forty-eight hours. Gelatin clear, granular deposit at the bottom and fine fragments of colonies on the surface.

*Potato.*—Fairly thick colonies, moist, dirty-white to yellow.

*Broth.*—Deposit of whitish granules in twenty-four to thirty-six hours, with fine, delicate pellicles on the surface, of the same color.

No turbidity.

*Milk.*—Acid in three days, coagulation, clot retracted to the bottom of tube at the end of eight days with the formation of an abundant clear serum.

Anaërobic media, especially sugar agar, show whitish lenticular colonies throughout the entire tube. On the surface the colonies are roundish and white. No fragmentation of the medium. Easily differentiated from "tetragenus" by the action on the gelatin and by the morphology.

1. Lindner: Die Sarcina-Organismen der Gärungsgewerbe. Inaug. Dissert., Berlin, 1888, p. 13.
2. Tissier: Recherches sur la Flore Intestinale des Nourrissons (Etat normal et pathologique). Paris, 1900, p. 60.

### SARCINA PULMONUM Virchow

Observed by Virchow[1] in the sputum of a case of phthisis. Similar sarcinas were subsequently found by Cohnheim,[2] Hauser,[3] and others. They have no etiological relation to disease, but represent the type of sarcina which occurs in cavities in the lungs.

**Morphology.**—Spherical cells arranged in characteristic packets.

**Cultivated** best at room temperature, with free access af atmospheric oxygen.

*Gelatin Plates.*—Colonies appear in three days as very fine white points which grow slowly and never become very large. Under low power they are characteristic. They are coarsely granular, irregular, with concentric peripheral layers. No liquefaction of gelatin.

*Gelatin Stab.*—Superficial growth only as a sharply defined, round, pearl-gray expansion which later becomes grayish-brown. It gradually becomes thicker with more or less folded glistening edges. Small delicate colonies along line of stab, gradually diminishing in number towards the bottom.

*Potato.*—Somewhat delicate expansion, limited to the line of inoculation.

*Urea Solution.*—Energetic ammoniacal decomposition.

1. Virchow: Virchow's Arch., 1856, ix, 557.
2. Cohnheim: Virchow's Arch., 1865, xxxiii, 157.
3. Hauser: Deutsches Arch. f. klin. Med., 1887, xl, 267.
   ———: München. med. Wchnschr., 1887, xxxiv, 545.

## GENUS 8. RHODOCOCCUS Zopf, emended Winslow and Rogers

Saprophytes. Cells in groups or regular packets. Generally decolorize by Gram. Growth on agar abundant with formation of *red pigment.* Glucose broth slightly acid, lactose broth neutral. Gelatin rarely liquefied. Nitrates generally reduced.

Type species *Rhodococcus rhodochrous* Zopf.
*Micrococcus roseus* (Bumm) Flügge.
" of Dantec.
" *carneus* Zimmermann.
" *cinnabareus* Flügge.
" *rosaceus* Frankland.

### RHODOCOCCUS RHODOCHROUS Zopf

Observed originally by Zopf.[1] Description from Migula.[2]

**Morphology.**—Spherical cells, about 1.1 $\mu$ in diameter when unstained, 0.5 to 0.6 $\mu$ when stained. Often arranged as diplococci or chains of 3 to 4 elements.

**Cultivation.**—*Gelatin Plates.*—After several days small, glistening, spherical, raised colonies, dark carmin-red in color, which aren ot more than 1 mm. in diameter, even after long periods of growth. Under low power the deep colonies are dark reddish-brown, spherical, with sharp smooth peripheries, almost homogeneous. The superficial colonies are circular with smooth sharp edges. Under magnification the color is like indigo-red or carmin.

*Gelatin Streak.*—Somewhat dry, glistening, dark carmin-rose expansion, several millimeters in breadth, showing numerous small warty protuberances as if the growth arose from several coalescing colonies.

*Gelatin Stab.*—Small superficial expansion on the surface of a rose color, delicate thread-like growth in the depths often made up of white colonies below and reddish colonies just beneath the surface. No liquefaction.

*Agar Slant.*—Carmin-red expansion becoming deep cinnabar-red in old cultures.

*Broth.*—Thick, smooth, moist, surface membrane, rose color with a reddish, powdery sediment.

Bergey[3] states that this species is Gram-positive and turns milk slightly alkaline. According to Zopf the pigment of this organism is a lipochrome, crystallizes in red aggregates and is characterized by a broad absorption band at F.

1. Zopf: Ber. d. deutsch. bot. Gesellsch., 1891, ix, 22.
2. Migula: p. 162.
3. Bergey, p. 75.

### MICROCOCCUS ROSEUS (Bumm) Flügge

Described as *Diplococcus roseus* by Bumm.[1] Named by Flügge.[2] Present description from Riley.[3]

**Morphology.**—Cocci of medium size, occurring for the most part singly and in pairs, but also in short chains and irregular groups. They measure 0.6 to 0.75 $\mu$ in diameter in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth rose pink, opaque, rather moist, with smooth, glistening surface; color is at first pale, but at room temperature deepens after a day or two to a bright rose-pink which may turn almost reddish at the margins; at incubator temperature, the pigment development is very slight; growth moderate in amount, regular, with smooth, sharp margins, raised, spreading very little from needle-track, except at bottom.

*Agar Stab.*—Filiform growth along needle-track; growth on surface spreading evenly, with regular edge, and smooth, glistening surface, slightly raised, opaque, rose-pink, deeper at edge.

*Agar Colonies.*—Round, with smooth edges, very slightly translucent, pink. Under low power—surface granular; margin smooth, sharply defined; color pinkish in middle, gray at edges.

*Dextrose Litmus Agar Slant.*—Growth smaller than on plain agar, color paler; strong acid formation.

*Dextrose Litmus Agar Plates.*—Colonies smaller than on plain agar; plates acid throughout in two to five days.

*Gelatin Stab.*—Filiform growth along needle-track, moderate growth on surface, somewhat deeper in color than on agar; liquefaction exceedingly slow, noticeable, generally, only as a decided cupping of the gelatin after about a month, though in one specimen the gelatin was completely liquefied after three months.

*Gelatin Colonies.*—Round, with smooth edges, pink, darker in the center.

*Broth.*—Liquid evenly clouded throughout, with a viscid sediment, white at first, later pink; no pellicle.

*Peptone Solution.*—Less growth than in broth.

*Litmus Milk.*—Becomes alkaline in about twelve to twenty days, later (twenty to fifty days) becomes decolorized and slowly digested, with slight acid formation.

*Potato.*—No growth.

*Blood-serum.*—Moderate amount of growth, rose-pink, moist, glistening; no liquefaction.

*Fermentation Tubes:*

*Dextrose Broth.*—Bulb faintly clouded; arm clear, with a small amount of sediment; no gas formation; no pellicle; reaction acid.

*Lactose Broth.*—Growth is in dextrose broth; reaction neutral.

*Saccharose Broth.*—Growth as in dextrose broth; reaction neutral.

**Remarks.**—Of the specimens cultivated by Riley one was of a deeper, more reddish shade than the others, but corresponded with them in all other respects, and was therefore classed with them. This organism corresponds in all characteristics with the *Diplococcus roseus* of Bumm, called *Micrococcus roseus* by Flügge, Migula,[4] and Chester.[5] It also corresponds with the *M. roseus* from the Bacteriological Collection of the American Museum of Natural History, New York, in morphology and cultural characters, though there is a slight difference in color, the latter being of a more yellowish-pink. According to Hucker this species can utilize ammonium salts as the sole source of nitrogen.

1. Bumm: Der Mikroorg. der. gon. Schleimhauterkrankungen. 1885, p. 25.
2. Flügge: Die Mikroorganismen. 2. Aufl. 1886, p. 183.
3. Riley: Air Organisms in Baltimore.
4. Migula: System der Bakterien. Jena, 1900, p. 176.
5. Chester: A Manual of Determinative Bacteriology. New York, 1914, p. 105.

### Micrococcus of Dantec

Found by Dantec in salted codfish which had decomposed with a red color and an offensive odor.

**Morphology.**—Micrococci from 3 to 5 $\mu$ in diameter, often showing binary division.

**Cultivation.**—*Agar Slant.*—Rapid development.

*Gelatin Plates.*—Small dish-shaped colonies of a reddish color, rarely measuring more than 1 mm. in diameter.

*Gelatin Stab.*—Slow development. Along line of inoculation the growth has a yellowish color. On the surface it is at first pale red then deep red.

Grows on dried codfish without pigment production except when associated with other organisms, especially with a liquefying coccus with which it is often found.

Pigment red.

### Micrococcus carneus Zimmermann

Found in water by Zimmermann.

**Morphology.**—Micrococci with a diameter of about 0.8 $\mu$ in irregular bunches.

**Cultural Characters.**—Grows best at room temperature. Very little growth at 30° to 33° C.

*Gelatin Plates.*—Deep colonies small, spherical, grayish-white. Superficial colonies slightly elevated, circular, grayish red to pale red. Under microscope circular disks with opaque reddish-gray centers surrounded by more transparent zones and again by paler zones. As the colonies become older the distinct zones disappear and the reddish-brown color fades out from the center ot the margin.

*Gelatin Stab.*—Fine, granular, white growth along line of inoculation at the end of about five days. On the surface a thin, circular, pale pink expansion with irregular outlines and about 3.5 mm. in diameter.

*Gelatin Slant.*—Flesh-red growth along line of inoculation, later acquiring a violet hue.

*Agar Slant.*—More abundant growth than on gelatin with the margins coarsely toothed.

*Potato.*—An abundant red growth.

### Micrococcus cinnabareus Flügge

Reported originally by Flügge.[1]

**Morphology.**—Large, spherical cocci often in form of diplococci, each of which is spherical. Frequently in bunches of three or four. Grows very slowly in *gelatin*. After four days deep colonies are punctiform, barely visible, while the superficial have an average diameter of 0.5 to 1 mm. After about eight days the superficial colonies are button-like and raised from the surface. Colonies at first light red in color, like sealing wax, later cinnabar-red. Under low power the deep young colonies are egg-shaped or lens-shaped, with sharp contours and dark

reddish-brown color. The superficial colonies appear light brown, with transparent edges, round or with somewhat irregular contour due to the protrusion of individual masses of cocci.

In *gelatin stab* cultures, isolated white colonies appear in the depths after four to five days while a fairly large button-like mass develops on the surface. This is first red, then cinnabar-colored. Gelatin not liquefied.

Very slow growth on *potato*.

Hucker[2] regards this species as the saprophytic non-liquefying micrococcus which produces a reddish pigment. Forms large irregular clumps of cells. Usually Gram variable. Produces moderate to abundant growth on agar slants. Generally curdles milk. Does not utilize ammonium salts as the only source of nitrogen.

1. Flügge: Die Mikroorganismen. 3. Aufl. 1896, ii, 177.
2. Hucker: The classification of genus micrococcus Cohn, New York State Agri. Exper. Sta., Tech. Bull. No. 102, June, 1904.

### MICROCOCCUS ROSACEUS Franklands

Observed frequently in the air by the Franklands.

**Morphology.**—Cocci variable in size, the largest being 2.5 $\mu$ in diameter and exhibiting a well-marked division.

**Cultivation.**—*Gelatin Plates.*—Small, shining, bright pink expansions, about pin-head in size. Under low power they are seen to be of a distinctly reddish tint, the edge being irregular but smooth. As the colonies approach the surface the irregularity diminishes.

*Gelatin Stab.*—Smooth, shining, pink expansion on the surface, whilst the needle-track below remains almost undeveloped. As the cultivation becomes older, the margin assumes a radiated appearance. Still older cultures frequently exhibit slight liquefaction.

*Agar Slant.*—Smooth, bright pink surface expansion.

*Broth.*—After nine days liquid clear, free from pellicle, and exhibits a pink deposit.

Frankland, G., and Frankland, P.: Philosophical Transactions. Roy. Soc., London, B. 1887, clxxviii, 257.

## GENUS 9. MOTILE COCCI

Micrococci, staphylococci, or sarcina forms, motile, usually by means of single flagella.

*Micrococcus agilis* Ali-Cohen.
" *tetragenus mobilis ventriculi* Mendoza.
" *citreus agilis* Menge.
*Sarcina mobilis* Maurea.
*Planosarcina ureæ* Beijerinck.
*Micrococcus flagellatus* Klotz.

### MICROCOCCUS AGILIS Ali-Cohen

Found originally in water by Ali-Cohen.[1] Regarded as a sarcina by Migula[2] who calls it *Planosarcina agilis.*

**Morphology.**—Micrococci about 1 $\mu$ in diameter arranged as single cells, diplococci and tetrads. According to Migula the cells taken from very thin agar show regular packets containing from 4 to 64 individuals.

**Motility** not marked in old cultures but definite in fresh cultures especially on Zettnow's agar where the movements are more active. Flagella may be demonstrated by special methods (Loeffler's) usually one to each cell, rarely two. They are ten times the width of the cell in length and usually curved.

**Stains** well by ordinary dyes. Gram-positive.

**Cultivated** best under aërobic conditions at temperatures between 15° and 20° C. Produces a dark rose-colored pigment which has a shade of cinnabar-red.

*Agar Slant.*—Glistening dark red expansion with overlapping edges.

*Gelatin Plates.*—Colonies appear as small gray points which assume a rose color in five days. The surface colonies form beautiful rose-colored drops. The deep colonies remain small and retain a grayish-red color.

Under the microscope the deep colonies appear as grayish-red almost spherical disks, the surface colonies are much the same, but larger and lighter with darker centers and almost colorless edges. No liquefaction.

*Gelatin Stab.*—Dark red, glistening, thick, circular expansion which gradually sinks into the gelatin. A thin whitish thread in the stab canal, rapidly disappearing in the depths. The pigment takes on a cinnabar-red color in gelatin.

*Potato.*—Slow growth of small punctiform colonies which gradually coalesce to a thick mass. More rapid growth on potatoes rendered alkaline.

*Broth.*—Slight turbidity and delicate, dirty, reddish-white deposit. Reddish ring-like growth along the wall of the tube, sinking to the bottom when agitated.

1. Ali-Cohen: Centralbl. f. Bakteriol. [etc.], 1889, vi, 33.
2. Migula: p. 275.

### Micrococcus tetragenus mobilis ventriculi Mendoza

Found by Mendoza[1] in stomach contents. Named *Planococcus tetragenus* (Mendoza) by Migula.[2]

**Morphology.**—Spherical cells enclosed in a transparent capsule and arranged usually as tetrads. The cell contents are finely granular.

**Motility** active in fluid media. The tetrads have a peculiar rolling motion as if turning about a hypothetical axis.

**Cultivation.**—*Development* slow on artificial media, an abundant intracellular substance practically always produced. Little anaërobic growth.

*Gelatin Plates.*—Superficial colonies white and circular with sharp edges. Under a stereoscopic microscope the contents are finely granular both in the centers which are darker, and on the lighter edges. No liquefaction. Old gelatin cultures have the color of sugar and give off an odor like skatol.

1. Mendoza: Centralbl. f. Bakteriol. [etc.], 1889, vi, 566.
2. Migula: p. 269.

### Micrococcus citreus agilis Menge

Described originally by Menge.[1] Named *Planococcus citreus* (Menge) by Migula.[2]

**Morphology.**—Micrococci arranged irregularly as single cells, diplococci, short chains, and bunches.

**Motility** active, especially when material from young cultures is examined in a favorable medium like broth. The individual cells move through the field in a direct path or turning at an angle. The diplococci show a to-and-fro movement about a hypothetical axis. The short chains and small bunches often swim actively about in the field. Single flagella attached to each cell can be demonstrated by Loeffler's method.

*Cultivated* best at 20° C. Growth slightly slower at 37° C. Growth only aërobic.

**Cultural Characters.**—*Gelatin Plates.*—Surface colonies large, elevated, yellow drops, finely granular under the microscope. Deep colonies small and regular. Surface colonies usually circular, but may become irregular as they get older. The gelatin assumes a yellowish color which arises as a turbidity from the surface colonies. Ordinary gelatin not liquefied. Gelatin of only 5 per cent. consistency may become somewhat softened.

*Gelatin Stab.*—Slight colorless growth in the depths. Slow surface growth with the formation of a flat, intensely yellow expansion.

*Slant Agar.*—Delicate, thin, pale growth along line of inoculation visible in about three days. This increases in breadth and thickness with the development of a definite yellow pigment. The edges of the growth are irregular and lighter in color than the central areas. Old agar cultures are extremely viscid. Water of condensation turbid, without surface film.

*Broth.*—Diffuse turbidity and thick yellow sediment. No surface film.

*Potato.*—Slow growth of an abundant light yellow expansion which becomes very viscid when old. The potato in the neighborhood of the yellow growth becomes grayish-blue.

*Milk.*—Yellowish pigmentation above, thick yellow sediment below. No coagulation.

Cultures kept in the dark grow just like those in the light, but show no pigmentation.

1. Menge: Centralbl. f. Bakteriol. [etc.], 1892, xii, 49.
2. Migula: p. 271.

### Sarcina mobilis Maurea

Found in ascitic fluid by Maurea.[1] Named *Planosarcina mobilis* by Migula.[2]

**Morphology.**—Spherical cells about 1.5 $\mu$ in diameter in pairs and tetrads. Packets are well defined in cultures in hay infusion.

*Motility* active, especially on fresh agar slants. Flagella can be stained by Loeffler's method, best on individual cells and on tetrads; with great difficulty on the sarcina forms. Each cell has apparently 2 flagella, much shorter than those on *Micrococcus agilis*. On the 8-celled packets the number of flagella is less than 2 to each cell.

Gram-positive.

Growth only at 22° C., not at 37° C.

**Cultural Characters.**—*Slant Agar.*—Whitish expansion in two days, later assuming a brick-red color. The pigmentation increases markedly as the cultures get older.

*Gelatin Plates.*—Colonies barely visible in three days as very small white points. Under the low power they are whitish, slightly granular, lens-shaped. Older colonies on the surface are larger, quite round, finely granular with central nuclei. Deep colonies are smaller, elliptical, darker and coarsely granular. Without nuclei. Liquefaction begins about the seventh or eighth day with the formation of a brick-red pigment.

*Gelatin Stab.*—Scanty growth along the line of inoculation, with a more abundant surface growth, which takes on a brick-red color. Liquefaction begins after fifteen to twenty days with the formation of a funnel-shaped excavation. Liquefaction proceeds slowly, the entire tube being liquefied only after two and a half months. The gelatin is then clear above with a yellowish-red sediment.

*Broth.*—Turbidity in forty-eight hours rapidly increasing. The fluid remains clear, while a yellowish-red deposit is formed at the bottom.

*Milk.*—Development after three days without coagulation.

*Potato.*—No growth.

1. Maurea: Centralbl. f. Bakteriol. [etc.], 1892, xi, 228.
2. Migula: p. 276.

### PLANOSARCINA UREÆ Beijerinck

Described by Beijerinck from urine.

**Morphology.**—Spherical cells 0.7 to 1.2 $\mu$ in diameter, arranged in packets of 4 to 8 and more individuals.

*Motility* active from peritrichic cilia which are easily stained. They are 7 to 8 times as long as the packets.

*Spores* spherical about 0.6 $\mu$ in diameter. They resist pasteurization and can be heated to 80° C. for ten minutes.

**Cultivation.**—Easily *cultivated* on infusion gelatin. Colonies flat, firm, dough-like, yellowish and of considerable size. No liquefaction.

This species contains considerable quantities of urease, splitting about 3 per cent. urea in five days.

Beijerinck: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1901, vii, 33.

### MICROCOCCUS FLAGELLATUS Klotz

Found in an epizoötic among rabbits and rats by Klotz[1] and described as a flagellate micrococcus.

**Morphology.**—A micrococcus larger than *Staphylococcus aureus* occurring most frequently in pairs, but also singly and rarely in short chains. Larger than *Micrococcus agilis* of Ali-Cohen. It may also appear in clusters of fours, especially in liquid media. Usually in pairs in the tissues and organs, not in chains.

*Motility.*—Zigzag and to-and-fro movements not covering much ground. The organisms seldom move from the center to the periphery of the field under the microscope. Eighteen-hour cultures from slightly alkaline blood-serum broth are most active.

**Staining Properties.**—The organism stains well by the ordinary dyes, best by carbolfuchsin and carbolthionin. They retain Gram's stain. The flagella may be stained by van Ermengem's method modified by Stephens.[2] A single flagellum is found attached to each coccus. When two cocci appear the flagella are attached to the poles opposite the adjacent surface. At times two flagella arise from the same point of the coccus. Each flagellum consists of a long lash-like process, very slender, many times the diameter of the organism in length.

The flagella are very delicate, easily broken and stain with great difficulty.

**Cultural Reactions.**—*Agar Slant.*—Luxuriant growth in fifteen hours on 1½ per cent. agar. The colonies develop from the streak in a grape-like fashion. Edges smooth, wavy, and slightly raised. Growth is white and glossy and does not penetrate the agar.

*Blood-serum.*—Growth thick, white, moist, and slimy with even edges, more abundant than in other solid media. Liquefaction occurs slowly, on about the tenth day.

Overheated blood-serum, turned grayish-brown, is liquefied more readily on the second or third day.

*Solidified Ascitic Fluid.*—Small, round, isolated colonies on the surface of the slant with liquefaction in three days.

*Potato.*—Slow growth visible only in forty-eight hours. More luxuriant after five days, appearing as a yellowish, dry, spreading colony with irregular heaped-up margins.

*Gelatin.*—Ten per cent., from nutrient broth slightly acid to phenolphthalein. Stab cultures give a granular growth along the line of inoculation. More vigorous in the upper part and spreading on the surface. Slow funnel-shaped liquefaction from above downward. Colonies on plates are small, grayish-white, with definite, slightly raised margins. At the end of a week small areas of liquefaction appear about the colonies.

*Broth.*—Diffuse cloudiness without pellicle in eighteen to twenty-four hours. In forty-eight hours a grayish ropy sediment which readily breaks up into fine flakes on shaking.

*Blood-serum Broth.*—Equal parts of blood-serum and glucose broth sterilized in the Arnold sterilizer make the best fluid medium. Cloudiness in twelve hours.

*Litmus milk* turned red within eighteen hours, then decolorized and coagulated. The coagulum becomes firm within twenty-four hours or less.

*Carbohydrates.*—No gas from glucose, lactose, or saccharose but growth in the closed arm of fermentation tubes.

*Optimum temperature* 37° C. Growth also at 20° C.

*Thermal Death-point.*—Not destroyed in broth cultures at 58° C. in five minutes, but destroyed at 60° C. in five minutes.

*Range of Reaction.*—Optimum growth with a reaction of 3 per cent. decinormal soda (to phenolphthalein). Growth in media with a reaction of 6 per cent. decinormal soda and with a reaction of 4 per cent. decinormal hydrochloric acid. No growth in media with 10 per cent. decinormal soda or 10 per cent. decinormal hydrochloric acid.

**Vitality.**—Transplants successful from cultures eight weeks old.

Growth anaërobic as well as aërobic.

**Pathogenic Action.**—Isolated originally from the blood and organs of rabbits and white rats during a chronic epizoötic in which loss of hair, emaciation, and loss of appetite were the most prominent features. At autopsy there was an increase of fibrous tissue in the liver. The cultures were virulent for rabbits, large doses (2 c.c. of twenty-four-hour broth culture) repeated on three successive days producing a chronic intoxication with an increase of fibrous tissue in the liver, and for guinea-pigs, 2 c.c. of a twenty-four-hour broth culture producing a chronic intoxication from which the animals died in fifty days.

1. Klotz: J. Med. Research, 1904, xi (n. s. vi), 493.
2. Stephens: Thompson Yates and Johnston Laboratory Reports, 1903, v, No. 1.

## GENUS 10. ANAËROBIC STAPHYLOCOCCI, DIPLOCOCCI, AND STREPTOCOCCI

Staphylococci, diplococci, and streptococci growing only under anaërobic conditions, usually pathogenic. Gram-positive or Gram-negative. Rare.

Arranged in chronological order:

*Micrococcus fetidus* Veillon.
*Krönig's anaërobic streptococcus.*
*Staphylococcus parvulus* Veillon and Zuber.
*Diplococcus reniformis* Cottet.
*Sternberg's anaërobic streptococcus.*
*Streptococcus anaërobius micros* Lewkowicz.
*Micrococcus gazogenes alkalescens anaërobius* Lewkowicz.
Silberschmidt's *anaërobic streptococcus.*
*Diplococcus magnus anaërobius* Tissier and Martelly.
*Micrococcus* A Grigoroff.
*Anaërobic coccus* of Gioelli.
*Streptococcus* Schwarzenbeck Gräf and Wittneben.
*Streptococcus* K Gräf and Wittneben.
*Diplococcus orbiculus* Tissier.
*Jungano's anaërobic staphylococcus.*
*Streptococcus putridus* Schottmüller.
*Staphylococcus aërogenes* Schottmüller.

Costa's *anaërobic streptococcus.*
*Staphylococcus asaccharolyticus* Distaso.
*Anaërobic streptococcus* Marwedel and Wehrsig.
Bloomfield's *anaërobic coccus.*
Adamson's *anaërobic diplococcus.*
*Micrococcus minutissimus* Oliver and Wherry.
Hall's *micrococcus.*
Thomson's *anaërobic diplococcus.*

### Micrococcus fetidus Veillon

This organism was first isolated by Veillon[1] from fetid pus in inflamed Bartholin's glands. It is apparently identical with the organism found by Rist[2] in suppuration of the ear, by Guillemot and Gottet in pulmonary gangrene, by Jeanin in putrid puerperal infections and by Hallé in the vagina and in inflamed Bartholin glands. The coccus found by Norris in liver abscesses was thought by him to be the same species. Menge and Krönig's anaërobic streptococcus may also be the same. According to Veillon it was also found by Ludwig in angina and in perinephritic phlegmon with fetid pus.

**Morphology.**—Small cocci, arranged singly as diplococci and in small masses. In cultures short chains of 3 to 4 diplococci occur. Occasionally elongated, almost bacillary with pointed ends (Veillon).

**Stains** readily by anilin dyes. Gram-positive.

**Grows** only under anaërobic conditions, feebly at 22° C. better at 37° C. Deep *agar* colonies small and round, whitish-gray. Uniform turbidity in *broth.* Slow, scanty development on *gelatin* which is not liquefied. All cultures give off very fetid gases, smelling like putrid blood or dental caries.

**Pathogenic** to rabbits and guinea-pigs on subcutaneous inoculation, producing cold abscesses in which the organism is present.

Norris' isolation grew best in neutral media. It formed flocculi and sediments in glucose broth plus hydrocele fluid or ascitic fluid. In neutral sugar agar it grew only in the depths, 1 centimeter below the surface. It formed 2½ to 3 per cent. acid in neutral glucose broth (calculated in terms of normal soda solution) but no gas. It rapidly lost its power of producing a fetid odor and was non-pathogenic.

1. Veillon: Compt. rend. Soc. de biol., 1893, xl, 807.
2. Rist: Centralbl. f. Bakteriol. [etc.], 1901, xxx, 287.

### Krönig's anaërobic streptococcus

Isolated by Krönig from the vaginal secretions of pregnant women by anaërobic methods and on meat extract media containing lactic acid. The anaërobic conditions were established by covering the media with layers of solid media, reducing substances, 5 per cent. grape-sugar, 0.3 per cent. sodium formate, or 0.3 per cent. indigocarmin being also added to the medium. The fluid agar was first inoculated with some of the vaginal secretions obtained aseptically, allowed to cool and then covered with a layer of sterile fluid agar.

*Cocci* in chains of 10 to 12 elements, exactly like the cocci found in puerperal fever. Gram-positive.

**Cultivated** easily in 5 per cent. *grape-sugar agar*, colonies being visible in twenty-four hours, about 2 cm. below the surface. Cultivated also in 5 per cent. *grape-sugar agar* containing lactose, sodium formate, or *indigocarmin.* The indigocarmin is reduced to colorless indigo by this streptococcus.

Only little growth is slightly *acid agar.* No growth in fluid media, *plain bouillon*, or *bouillon* containing grape-sugar or milk sugar. No coagulation of *milk.* Optimum temperature 28° to 40° C. No growth under 25° C. No aërobic growth.

Non-virulent to rabbits.

Similar staphylococci, groups made up of thick cocci, Gram-positive, were also isolated by Krönig. Cultural characters like those of the anaërobic streptococci.

Krönig: Centralbl. f. Gynäk., 1895, xix, 409.

### STAPHYLOCOCCUS PARVULUS Veillon and Zuber

Obtained originally by Veillon and Zuber[1] from fetid pus in appendicitis. Apparently the same species was obtained by Guillemot in pulmonary gangrene, by Cottet and Jungano in urinary tract infections, by Rist in chronic middle ear disease, by Lainer in septic diphtheria, and by Heyde in acute osteomyelitis. The organism found by Lewkowicz in the mouths of very young children and named *Micrococcus gazogenes alkalescens anaërobius* is the same or closely related. Extensively investigated by Ozaki[2] who obtained his cultures from material about the teeth in normal persons.

**Morphology.**—Very small cocci, 0.3 to 0.4 $\mu$ in diameter, arranged as single individuals, as diplococci, as short chains of 3 to 5 elements, and as bunches like staphylococci. The cells may be somewhat flattened, especially in the diplococci.

**Stained** feebly with methylene-blue, best with carbolfuchsin. Gram-negative. Claudius-negative.

**Cultivated** only as an obligate anaërobe, best at 37° C., slowly at 22° to 23° C. Develops well on the majority of media, but grows best on neutral or weakly alkaline media. Growth favored by grape-sugar. Organisms viable at first for fifteen to twenty days in sugar agar at 37° C., later only four to five days (Ozaki).

*Agar Plates.*—Surface colonies small, very fine points, very transparent, and slowly disappearing. Deep colonies well defined and limited, yellowish, granular at first. A small amount of gas is evolved which cracks the agar feebly, and is very fetid (Veillon).

*Stab Cultures in 1 per cent. grape-sugar agar* show in twenty-four hours at 37° C. a fairly good growth along the line of inoculation, at first in the form of a homogeneous whitish thread without side branches (Ozaki). Later, short extensions appear from this line. Under a hand lens the thread is finely granular and pale brown. In eighteen to thirty hours gas bubbles appear in the medium near the line of inoculation, and later the agar is fragmented. Frequently a turbid fluid collects on the top of the medium.

*Plain agar stabs* show a similar thread-like whitish growth which is not so abundant, with little or no gas formation.

*Plain agar shake cultures* show in the depths at 37° C. many small, barely visible or slightly larger, whitish points. Under low power the larger colonies are round or roundish, frequently obtuse-angled with smooth edges, pale brownish-yellow by transmitted light. After forty-eight hours they measure $\frac{1}{2}$ mm. in diameter but many may be smaller. Under low power they are round or oval, heart-shaped, obtuse-angled, sharply contoured, very finely granular, pale brownish-yellow by transmitted light. After four days the colonies are somewhat larger.

*Sugar agar shake cultures* (1 per cent. sugar) show colonies like those in plain agar, but much gas is evolved.

*Surface colonies* on *agar slants* in an atmosphere of hydrogen appear in four days at 37° C. They are small grayish-white points which gradually enlarge. The water of condensation is clear and contains a small whitish sediment. After eight days at 37° C. the colonies are unequal in size, usually isolated, grayish-white like dew-drops. They resemble streptococcus colonies. The largest at $\frac{1}{2}$ mm. in diameter, the smallest barely visible. The large colonies are round or oval, raised, gray-white, translucent, and succulent-looking. With a hand lens they appear pale brown, transparent, with slightly uneven edges, frequently with darker centers and 1 to 2 concentric cross-striations. The water of condensation remains clear and contains a fairly large quantity of whitish, flaky, or granular sediment which on shaking produces a diffuse turbidity and granular flakes. Similar colonies on *plain agar* in an atmosphere of hydrogen (Ozaki).

*Deep gelatin colonies* grow slowly; after a week appearing as small, opaque, granular, brownish masses, with smooth or slightly undulating borders (Veillon). Older colonies are browner, more opaque, mulberry-like. No liquefaction.

*Broth.*—Turbidity and dust-like sediment. Abundant growth in twenty-four hours at 37° C. in broth containing pieces of organs. These turn slightly blackish and the broth becomes cloudy (Ozaki).

Moderate gas evolution in meat extract with 1 per cent. grape sugar added, in an atmosphere of hydrogen. After four days there is a slight whitish sediment. After eight days this is more abundant, pale yellow-white, dusty, or granular. On shaking this breaks up into smaller particles without especially clouding the fluid. Sugar gelatin covered with a layer of

agar shows a similar reaction. In liquefied gelatin at 37° C., a small quantity of yellowish-white granular sediment collects. The gelatin is not clouded and quickly solidifies on cooling. Gas collects between the gelatin and the agar, its quantity corresponding to about $\frac{1}{10}$ of the gelatin.

*Milk.*—Slight growth, few gas bubbles, no coagulation.

*Potato.*—Invisible growth in an atmosphere of hydrogen. Good growth in *potato broth.* Gas formation begins in twelve hours at 37° C. and lasts about two days. The broth becomes cloudy, the potato does not change.

Poor growth in an atmosphere of $CO_2$. On sugar agar it is barely visible in four days as gray-white colonies, which increase but little in size. Under low power they are round or oval, raised, glistening, and moist. No sediment in water of condensation.

Gas production in dextrose, levulose, maltose, saccharose, lactose, dextrin, glycerin, starch, and mannite. It reaches its maximum in eighteen hours and lasts sixty hours. Gas usually very fetid. Some cultures not stinking (Ozaki). Indol and $H_2S$ not produced. Litmus agar decolorized. No hemolysis.

**Pathogenic Action.**—Subcutaneous injection of large doses produces abscesses in mice and guinea-pigs. They are lentil- to pea-sized, contain thick yellow pus which does not stink. In rabbits subcutaneous injection produces an infiltration but not abscess formation.

1. Veillon and Zuber: Arch. de. méd. exper. et d'anat., 1898, x, 517.
2. Ozaki: Centralbl. f. Bakteriol. [etc.], 1912, lxii, 76.

### Diplococcus reniformis Cottet

Isolated originally by Cottet[1] from abscesses of the urinary tract, from the pus from a case of pyonephrosis and from the urine in cystitis. Found by Oliver and Wherry[2] in the vaginal pus in a case of vulvo-vaginitis in a child.

**Morphology.**—Biscuit-shaped or reniform diplococci slightly smaller than the gonococcus, consisting of 2 individuals joined by their flat surfaces and separated by a very narrow interval. In cultures it retains a uniform size without showing the swollen involution forms which appear early in gonococcus cultures.

**Stains** by usual anilin dyes. Gram-negative, but more slowly decolorized than the gonococcus. It has a tendency to remain slightly stained.

**Cultivated** anaërobically by Cottet in Liborius tubes and by Oliver and Wherry in *ascites agar* by anaërobic methods. It grows well in deep *sugar agar* at 37° C. (Cottet). At the end of thirty-six to forty-eight hours fine colonies appear in the zone deprived of air, looking like bluish-white points. Under low power (Leitz obj. 2, ocular 3) these colonies look like small roundish masses, yellowish in color, semitransparent, centers somewhat opaque, borders smooth, but finely dentated. Older colonies moruloid. No gas. The cultures have the odor of rancid butter. The colonies retained their vitality for a long time and could be transferred after five to six weeks.

In stab cultures in *ascites agar* growth ceased at a point 6 mm. below the surface (Oliver and Wherry). The organisms died on exposure to the air.

Turbidity in *bouillon* in forty-eight hours. A flocculent deposit forms and the liquid clears like a bouillon culture of streptococci.

On the surface of *agar* cultivated by Roux's method colonies appear in forty-eight hours. They are round, fine, whitish, slightly bluish by reflected light like streptococcus colonies.

No growth in *gelatin, sugar gelatin, Loeffler's blood-serum, plain agar, milk* or *potato.* No fermentation of destrose, levulose, galactose, saccharose, maltose, lactose, or mannite.

Indol produced in sugar-free broth containing sodium phosphate and ascitic fluid.

Subcutaneous injections in guinea-pigs produce circumscribed abscesses.

1. Cottet: Compt. rend. Soc. de biol., 1900, lii, 421.
2. Oliver and Wherry: J. Infect. Dis., 1921, xxviii, 341.

### Sternberg's anaërobic streptococcus

Isolated by Sternberg from the sputum in a case of actinomycosis of the lung.

**Morphology.**—Fairly long chains made up of round or oval, fairly plump cocci of various sizes, in general like a streptococcus but 2 to 3 times as large. Gram-positive.

**Cultivated** only under anaërobic conditions.

*Agar Slant.*—Delicate fine expansion made up of small discrete colonies.

*Agar Stab.*—Scanty development.

*Sugar Agar Plates.*—Small colonies about the size of a pin-head, microscopically dark brown to black, with irregular surface and smooth edges from which many prolongations extend peripherally.

*Plain Agar Plates.*—Scanty development in the form of small colonies, microscopically appearing granular, with wavy borders, and radial striations in the periphery.

*Bouillon* remains clear, with a scanty sediment.

*Sugar Bouillon.*—Abundant sediment with small granules along the wall of the test-tube.

*Gelatin Stab.*—Fine veil-like turbidity in the depths at room temperature, with small granules about the size of pin-heads.

*Loeffler's Blood-serum.*—Small, slightly raised, moderately moist colonies, pin-head in size.

*Potato* slightly acid (not neutralized), shows no growth or very small white granules. Microscopically potato covered with organisms, which could be transferred for at least a month.

Abundant development in *sugar agar stab* and *sugar agar broth* under anaërobic conditions. In the stab the growth was made up of granules. In the broth (covered with paraffin) an abundant growth of small granules appeared in twenty-four hours, so that the bottom of the flask was covered with a thick layer, with streaks running up the wall. The isolated granules were large, the size of millet seeds or larger, and occasionally coalesced to larger clumps.

Subcutaneous injections in rabbits produced localized nodules made up of granulation tissue.

Sternberg: Wien. klin. Wchnschr., 1900, xiii, 551.

### STREPTOCOCCUS ANAËROBIUS MICROS Lewkowicz

Described by Lewkowicz from the mouths of nurslings.

Very small cocci 0.25 to 0.4 $\mu$ in diameter, often elongated or lanceolate. Many involution forms. Bacillary forms in sugar agar. Usually arranged as diplococci or short chains. Gram-positive.

**Cultivation.**—*Grows* only at 37° C. as a strict anaërobe. Lives two to three weeks in cultures. Killed at 60° C. in fifteen minutes.

In *deep sugar agar* growth appears in two to three days, 10 to 15 mm. below the surface, made up of small colonies, 0.05 to 0.07 mm. in diameter. Microscopically they are round, finely granular and transparent or with the short filamentous prolongations. Isolated colonies are 2 to 3 mm. in size under the microscope, whitish, round or oval, opaque, often with similar fine filamentous prolongations. Rarely colonies are 0.6 to 0.8 mm. in size. Surface colonies when isolated are 0.25 to 0.3 mm. in diameter after forty-eight hours, later 0.8 mm. in size. They are round, transparent, grayish, slightly elevated, microscopically finely granular.

Turbidity in *broth* with an abundant powdery deposit.

No coagulation of *milk*.

No gas from cultures, but a well-marked odor.

Practically non-pathogenic.

Lewkowicz: Arch. d. méd. expér. et d'anat. path., 1901, xiii, 633.

### MICROCOCCUS GAZOGENES ALKALESCENS ANAËROBIUS Lewkowicz

Obtained by Lewkowicz from the mouth of nurslings. Resembles *Staphylococcus parvulus* of Veillon and Zuber.

**Morphology.**—Cocci 0.3 to 0.7 $\mu$ in diameter, round when isolated, flattened when diplococci. Grouped in diplococci, tetrads, and masses.

Stains by usual dyes. Gram-negative.

**Cultivation.**—*Grows* as a strict anaërobe at 37° C. No growth at 15° to 20° C. Dies out in cultures in six days. Rarely lasts twenty-four days. Killed at 60° C. in five minutes.

*Deep sugar agar* colonies appear in twenty-four hours, small, punctiform, grayish, transparent with numerous bubbles of gas. Eventually the agar is completely fragmented with an expression of fluid. Colonies measure 0.01 to 0.05 mm. in diameter. Under the microscope they are finely granular with smooth edges. With light seeding colonies may be 0.5 to 1.0 mm. in diameter, round or lenticular, grayish-white with a shade of olive. Rarely isolated colonies may be 4 mm. in diameter. Surface colonies in thirty-six to forty-eight hours 0.5 to 0.9 mm., after five days 1.5 mm. in diameter. They are round, dome-shaped, grayish, transparent, and reflect light from the surface. Under the microscope they are finely granular, with opaque centers and large granules toward the periphery.

*Broth* shows turbidity after twenty-four hours and a very fine sediment slowly deposited.
Abundant gas in *sugar broth*.
No coagulation of *milk*, but gas production.
Delicate, colorless transparent growth on *potato*.
No growth in *gelatin*.
No change in reaction of media.
Gas not fetid and has practically no odor.
Non-pathogenic except for slight edema and inflammation at point of inoculation.

Lewkowicz: Arch. d. méd. expér. et d'anat. path., 1901, xiii, 633.

### Silberschmidt's anaërobic streptococcus

Isolated by Silberschmidt from a fatal case of gas gangrene in association with streptococci, staphylococci, and bacilli.

In the pus it appeared as a typical streptococcus in short and long chains. In cultures the organisms were small and usually stained with difficulty. In certain cultures, as in anaërobic surface agar, the cocci stained typically, in short curved chains, here and there enclosed in a capsule. Usually Gram-positive.

**Cultivated** only as an obligate anaërobe, the growth resembling that of a streptococcus or diplococcus. More abundant in *broth* than in *sugar broth*, after two days giving a sediment and a fine deposit along the walls. The cultures have a marked unpleasant odor.

*Agar Slant.*—Small, sharply limited, transparent colonies, punctiform to pinhead in size. Abundant growth in water of condensation.

*Agar Stab.*—Growth in the depths with wavy edges. No isolated colonies.

*Milk.*—No clotting.

*Gelatin.*—No growth.

*Non-pathogenic* to mice and guinea-pigs.

Silberschmidt: Ztschr. f. Hyg., 1902, xli, 427.

### Diplococcus magnus anaërobius Tissier and Martelly

Found by Tissier and Martelly in decomposing meat.

Large Gram-positive coccus, isolated or in pairs with flattened edges. Many involution forms in old cultures.

*Cultivated* as a strict anaërobe growing at 22° C. or at 37° C. Small colonies on glucose agar 1 to 2 mm. in diameter at the end of four to five days. Opaque centers, thin edges, granular. *Gelatin* colonies granular and flocculent. No change in *milk*. Turbidity in *broth*. Fibrin not digested. No liquefaction of *gelatin*. No acid from *sugars*. Converts proteoses to ammonium carbonate and ammonia. No indol. Converts urea into ammonium carbonate. Favors the growth and vitality of *Bacillus perfringens* (*B. Welchii*).

Tissier and Martelly: Ann. d l'Inst. Pasteur, 1902, xvi, 865.

### Micrococcus A Grigoroff

Found by Grigoroff in appendicitis.

Small cocci, isolated or in groups, staining by ordinary dyes. Gram-positive.
Colonies appear on third day in *glucose agar*, round, yellowish, and regular.
In *glucose bouillon* turbidity on second day with a whitish deposit at the bottom of the tube and an acid reaction.
*Milk* acidified and coagulated.
Colonies appear on third or fourth day in *sugar gelatin*. No liquefaction.
Acid in glucose, maltose, lactose, levulose, and sorbite.
Cultures not fetid like *Micrococcus parvulus* of Veillon and Zuber.
Non-pathogenic.

Grigoroff: Contribution a la pathogénie de l'appendicite. Thèse de Genève. 1905, p., 74.

### Coccus anaërobius of Gioelli

Described by Gioelli from a periuterine abscess.

Small coccus, diplococcus and small clumps, individuals smaller than *Staphylococcus aureus*. Stains feebly with dyes only after long exposure.

Gram-negative.

An obligate anaërobe. Colonies in *deep sugar agar*, at end of two to three days, small, whitish, round, surface granular, with an abundant production of fetid gas. Active fermentation of glucose, lactose, and maltose, less energetic of saccharose and mannite. Growth in *milk* without coagulation.

No indol.

Slightly pathogenic for guinea-pigs, rabbits, and rats.

Gioelli: Boll. Acad. med. di Genova, 1907.

### Streptococcus Schwarzenbeck Gräf and Wittneben

Described by Gräf and Wittneben from a skin abscess suspected of being actinomycosis.

Films from the purulent material stained by Gram revealed regular cocci in diplococci or short chains, together with Gram-positive rods. Aërobic cultures at 37° C. in twenty-four hours uniformly negative except for a barely visible expansion on Loeffler's serum which on the next day showed small pure white isolated colonies. Anaërobic cultures more successful, good growth being obtained in broth, milk, deep agar tubes, etc.

**Morphology.**—Cocci or short oval rods like those seen in ordinary streptococcus cultures, the cocci being usually in chains. In neutral bouillon the chains were composed of 30 to 50 elements, in alkaline bouillon of only about 8 elements. The chains were frequently wound and tangled (like Kurth's *Streptococcus conglomeratus*). The individual elements were smaller than the ordinary *Streptococcus pyogenes*.

**Stains** best by Gram, less well with methylene-blue, somewhat better with dilute carbol-fuchsin (1 to 10). No Neisser granules. Not acid-fast. No capsules. No flagella.

Optimum temperature 25° C. No growth at 22° C., some growth at 25° C.

Vitality on the surface of media about two to three weeks, in condensation water of Loeffler's serum about eight weeks.

**Cultivation.**—*Cultivated* best under anaërobic conditions. Some aërobic development on media containing serum and sugar. Very slight growth on *litmus-lactose-agar* of v. Drigalski and Conradi, due possibly to the nutrose present. On *agar* which contains only serum or only sugar no superficial growth under aërobic conditions. Deep *agar* shake cultures show above a colony-free zone, 15 mm. deep, sharply defined, then a zone 7 mm. deep where the agar is thickly studded with colonies, then again a zone about 6 mm. deep with no colonies, and finally a zone of abundant development at the bottom of the tube. This streptococcus seems to develop in two different concentrations of oxygen.

All surface colonies stick closely to the media, leaving a defect in the medium when removed.

**Cultural Characters.**—*Neutral Agar Plates.*—No superficial colonies under aërobic conditions. Deep colonies in seventy-two hours under a magnification of 60 diameters are black-brown, flaky with yellow, with rosette-like toothed edges. After five days a yellow honeycomb-like zone appears about the colonies. In an atmosphere of hydrogen, yellowish, round, finely granular, superficial colonies with sharp edges developed.

*Agar Stab.*—Definite development in the depths in twenty-four hours; delicate, thread-like, not characteristic. In forty-eight hours very faint superficial expansion.

*Deep Agar Shake.*—Upper zone, 9 to 10 mm. deep, free of colonies; below, round, irregular, whitish colonies in forty-eight hours.

*Acid Agar Plates.*—No growth under aërobic conditions. In an atmosphere of hydrogen the colonies appear under a magnification of 60 diameters as coarsely granular, round expansions, with irregular, coarsely granular edges.

*Deep Acid Agar Shake.*—Moderate development in the depths only with rich inoculations. Above, a colony-free zone 15 mm. deep.

*Grape-sugar Agar.*—Growth like that in neutral agar, but somewhat more abundant.

*Glycerin Agar.*—Growth like that in grape-sugar agar.

*Serum Agar* (1 to 5).—No superficial colonies under aërobic conditions. Deep colonies appear in forty-eight hours, whitish with irregular edges. With a low magnification (60 diameters) they are blackish-brown, rosette-like, flaky. Growth in atmosphere of hydrogen like that in neutral agar.

*Serum Agar Stab.*—Growth like that in neutral agar. Shake cultures show the typical 4 zones.

*Grape-sugar Serum Agar.*—Under aërobic conditions superficial colonies round, succulent, yellowish-white, size of pin-heads. Under low magnification (60) blackish-yellow, granular, with fairly sharp edges. With a magnification of 500 diameters cocci in chains appear, long and oval.

*Grape-sugar serum agar shake* cultures show whitish colonies arranged in zones, most abundant in the depths.

*Milk Agar* (1 to 1).—Superficial colonies in forty-eight hours sparce, snow-white, with faint zones. Under low power (60) black, round, edges flaky and irregular. Better development in the depths.

*Milk Agar Shake Cultures.*—Colony free zone 5 mm. above; below, snow-white, irregular, roundish colonies, larger than those of *Streptococcus pyogenes.*

*Blood Agar* (1 to 10).—Superficial colonies barely visible in forty-eight hours, very delicate, punctiform, whitish. No hemolysis.

*Loeffler's Serum.*—Punctiform pure-white colonies in forty-eight hours. In seventy-two hours up to 1 mm. wide, not definitely round, edges clearly irregular, central portions raised, button-like.

*Drigalski-Conradi Agar.*—Trace of growth in three days with rich seeding. Colonies reddish-white or light red with dark red centers by transmitted light. Same type of growth when medium contains no crystal violet.

*Drigalski-Conradi Agar with Serum Added.*—Superficial colonies in forty-eight hours reddish-white, somewhat succulent, not definitely round, edges indented, narrower by transmitted light, with lighter reddish-white edges, middle portion bluish-red. With a magnification of 60 diameters the colonies are round, rosette-like, with light edges. A magnification of 500 shows thick oval rods in chains and Y formation as well as chains of cocci, containing up to 20 elements. Better growth in the depths than on the surface. Medium red throughout.

*Potato.*—No visible growth.

*Gelatin.*—No growth at 22° C. At 25° C. no superficial growth. In the depths, colonies appear under low magnification (60) black, flaky, rosette-like. Periphery yellowish. No liquefaction.

*Gelatin Stab.*—Thread-like growth, later showing white button-like masses. Growth in gelatin at 37° C. good under anaërobic conditions. Gelatin fluidified by a ferment. More abundant growth in serum gelatin, only at 37° C.

*Bouillon* freshly boiled shows slight development in twenty-four hours under both aërobic and anaërobic conditions, more abundant in forty-eight hours. Granular masses along the glass, sinking to the bottom, bouillon clear, yellowish-white granular sediment. In bouillon not covered with paraffin, growth along the glass begins 1 cm. below the surface.

*Grape-sugar Bouillon.*—Somewhat more abundant growth.

*Acid Bouillon.*—No growth.

*Alkaline Broth.*—More abundant growth than in neutral broth.

*Neutral broth* under a magnification of 500 shows chains of 20 to 30 elements beautifully wound. Alkaline broth shows chains with only 8 elements and ovoid rods in V forms.

*Peptone Water.*—Faint growth. No indol.

*Potato Bouillon* (Wrzosek).—Growth like that of *Streptococcus pyogenes.*

*Milk.*—Clotted in four days to fairly firm masses. Under a magnification of 500 diameters short chains and diplococci appear. No clotting at 22° C.

*Litmus Milk-whey.*—Reddening begins in seven days, outspoken in four weeks, not increasing, slight sediment.

*Barsiekow's Milk Sugar Medium.*—Reddening, gradually becoming deeper, marked in six weeks, opalescent by reflected light.

*Barsiekow's Grape-sugar Medium.*—Marked reddening in forty-eight hours, strong in fourteen days, somewhat opalescent in reflected light. No clotting. Clotting in one and a half to three months.

**Pathogenic Action.**—Non-virulent to mice, guinea-pigs and rabbits by intraperitoneal inoculation. Subcutaneous inoculation produced in guinea-pigs nodules up to cherries in size, disappearing after six to eight days. On incision the nodules were found to contain pus. Similar nodules produced in mice, lentil- or pea-sized. Abscesses also produced in rabbits.

Gräf and Wittneben: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1907, xliv, Orig., 97.

### Streptococcus K of Gräf and Wittneben

An anaërobic streptococcus cultivated by Gräf and Wittneben from the pus of a brain abscess. In its general characters it resembles *Streptococcus Schwarzenbeck.*

Gram-positive, very small cocci in chains and in small masses. In *deep agar shake* cultures of *neutral*, *grape-sugar*, and *glycerin agar*, a colony-free zone 5 to 17 mm. appears above, and below this a zone of thickly distributed colonies. Better growth on media containing grape sugar and on acid media. Considerable acid produced in cultures, 1 c.c. of a twenty-four-hour grape sugar broth growth requiring 0.5 c.c. $\frac{1}{10}$ normal sodium hydrate for neutralization, corresponding to 0.3 c.c. 1 per cent. acetic acid. *Milk* acidified and clotted in forty-eight hours. Outspoken reddening of *litmus milk-whey*, Barsiekow's *dextrose* and *lactose* media, and *litmus nutrose lactose agar*. Colonies cling to the surface of solid media, leaving a defect in the medium when removed.

Non-pathogenic to white and gray mice, guinea-pigs, and rabbits by subcutaneous and intravenous inoculation.

No hemolysis on blood agar plates.

Grows only sparsely under aërobic conditions. Grows well in an atmosphere of hydrogen.

Gräf and Wittneben: Centralbl. f. Bakteriol. [etc.], 1907, xliv, 97.

### Diplococcus orbiculus Tissier

Found by Tissier in the stools of young infants. Large diplococcus joined by flat surfaces, two or three times as large as the gonococcus.

Stains by ordinary anilin dyes. Gram-negative. Obligate anaërobe, grows only at 37° C. Lives in cultures only six to eight days. Deep agar colonies in thirty-six to forty-eight hours, large, lenticular, very regular, slightly thick, whitish, and almost transparent. No gas. Turbidity and granular deposit in fluid media.

No coagulation of *milk*.

No action on cooked *white of egg*.

Acidity greater in glucose than in lactose. No acid with saccharose.

Attacks proteoses without producing indol.

Non-pathogenic.

Like *Diplococcus reniformis* of Cottet which is smaller, pathogenic, and gives a disagreeable odor in cultures.

Tissier: Ann. de l'Inst. Pasteur, 1908, xxii, 189.

### Jungano's anaërobic staphylococcus

Isolated by Jungano from a case of cystitis and from a case of gangrenous inflammation of the perineum, by Jungano and Distaso from various infections of the urinary apparatus as well as from the normal urethra. Found by Distaso in the dejecta. Description from Jungano and Distaso.

Very small cocci staining well with anilin dyes and by Gram.

*Grows* well in *glucose agar*, colonies appearing in forty-eight to sixty hours, small and shiny; when grown they are round or biconvex, thicker in center than in periphery.

*Broth* turbidity in thirty-six hours. The broth gradually clears, being quite limpid after fifteen days.

Grows at 22° C. In *gelatin* after five to six days colonies are small, round, regular, transparent, and shiny. No liquefaction. No gas from cultures. No action on sugars.

No growth in *milk*. No action on *white of egg*.

Cultures remain alive two months.

Pathogenic for guinea-pigs and rabbits.

Jungano and Distaso: Les Anaérobies. Paris, 1910, p. 194.

### Streptococcus putridus Schottmüller

Obtained by Schottmüller[1] from a great variety of cases including otitis media, meningitis, empyema, endometritis, salpingitis, peritonitis, etc. Apparently the same organism was isolated earlier by Menge and Krönig[2] from pathological conditions in the puerperium in which stinking pus is present.

**Morphology.**—Diplococcus forms and longer and shorter chains of streptococci from the infected tissues. In artificial media short and long twisted chains made up of oval elements arranged in pairs as diplococci. In old cultures the organisms appear as rods and granules of very different sizes.

**Stains** with usual anilin dyes. Gram-positive.

**Grows** only as an obligate anaërobe under very complete anaërobic conditions. These conditions are best obtained in deep agar, either in stabs or shake cultures. The addition of reducing substances favors the growth of the organisms. Cultivated well also in broth containing blood where apparently the organisms at the bottom are protected from oxygen. Ordinary anaërobic methods, such as the use of pyrogallic acid, do not suffice for cultivation. No satisfactory growth in ordinary broth. Cultivated on blood agar plates in an atmosphere of hydrogen. Growth best obtained in shake cultures in sugar agar containing blood, the blood being added to the melted agar at 45° C. The colonies develop in about twenty-four hours and the agar is full of gas bubbles.

*Grows* best at 37° C. No growth at 20° C. Vitality poor. Transfers successful for only fourteen days.

On plain *agar plates* in an atmosphere of hydrogen, the colonies are small and gray, like those of the ordinary streptococcus.

In *agar stabs* in twenty-four to forty-eight hours the colonies develop in the anaërobic zone, small, grayish-yellow, whetstone in shape. Similar colonies in shake agar cultures.

Good growth in indigo and neutral red agar, the latter showing decolorization and fluorescence.

Gas formation does not occur in ordinary agar or sugar agar, but does occur in blood agar. The gas is $H_2S$, stinking and inflammable.

Colonies in *blood agar* small, gray-white, resembling muscle trichinæ. Later they are pin-head in size. The cultures give off a peculiar, disagreeable odor. Colonies on *blood agar* plates porcelain white, the size of pin-heads. Deep colonies are glistening white. No hemolysis. No gas in sugar agar.

Good growth in *milk*. No clotting.

The putrid odor is especially perceptible in blood bouillon cultures when the blood pigment takes on a peculiar light red shade. The spectroscope shows $H_2S$. After about ten days the bouillon cultures are black.

Blood from infected cases and pus also has the disagreeable odor.

Non-pathogenic to rabbits and guinea-pigs.

**Remarks.**—This organism is regarded by Schottmüller as the causative agent in fetid infections in which stinking gases develop such as the thrombophlebitic form of puerperal sepsis (*Putrescentia uteri*), secondary local purulent processes such as pyosalpinx, abscesses in Douglas' pouch, etc. It is frequently the cause of lung gangrene, causing a destruction of lung tissue and a disagreeable odor.

1. Schottmüller: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1910, xxi, 450.
2. Menge and Krönig: Monatschr. f. Geburtsh. u. Gynäk., 1899, ix, 703.

### STAPHYLOCOCCUS AËROGENES Schottmüller

Isolated by Schottmüller[1] in puerperal infections, endometritis, salpingitis, purulent peritonitis, puerperal sepsis (chronic pyemia).

**Morphology.**—Cocci of various sizes in cultures and in pus not to be distinguished from *Staphylococcus aureus*, round, about 0.8 $\mu$ in diameter, in typical grape-like clusters. Also diplococci, occasionally in rows of 6 to 10 elements, the cocci being joined in rows in the direction of their plane of segmentation and thus differentiated from streptococci. Also in tetrads almost like a sarcina. These are especially found in blood agar cultures.

**Stains** readily by anilin dyes, the majority being Gram-positive. A few, however, are Gram-negative, take the counterstain (safranin) and thus look like gonococci.

**Cultivated** only anaërobically. Growth in ordinary agar to which blood or pus is added only for one generation. This is brought about by the protein materials, which can also be supplied by serum, ascites fluid, or pleural fluid. Further transfers possible, however, only under anaërobic conditions.

Cultures on *blood agar plastilin plates* (Schottmüller[2]) after twenty-four hours at 37° C., show an expansion of dark gray colonies of various sizes, some barely visible, some pin-head in size. They have a glistening surface and are slightly vaulted or arched. Others have navel-like centers, the outer rims slightly raised and wall-like. The colonies show considerable variation in color. After three days the colonies are somewhat larger, but their growth is ended. Protected from drying, the organisms remain alive for several weeks.

In *grape-sugar blood agar shake* cultures (blood 3 parts, agar 10 parts) after two days small gray colonies are visible when the seeding is not too heavy. In cultures four to five days old a few gas bubbles appear; with heavy seeding they may fragment the agar. Later generations often have a diminished power of gas formation. In *serum glucose sugar agar* in twenty-four to forty-eight hours fine, whetstone, whitish-yellow colonies appear, with gas bubbles. Further transfers are without result.

*Serum neutral red agar* shows well-marked colonies, gas bubbles and a change of the red color to olive-green. No growth in neutral red agar without serum or blood.

Good growth also in milk agar (2 to 3). While the colonies are not visible gas bubbles are produced by the growth.

*Deep serum agar stabs* show in twenty-four hours a thick streak along the line of inoculation, made up of countless gray colonies. The upper zone, 1.5 cm. deep, is always free from growth because of the inhibiting action of the atmospheric oxygen.

On *blood agar, serum agar*, and *milk agar plates* colonies appear in twenty-four to forty-eight hours. They are small, dewdrop, translucent, grayish-yellow by reflected light, glistening, smooth, not over pin-head in size. Serum agar plates valuable for continued cultivation.

In *blood sugar broth* (2 to 5) a slight turbidity appears in the broth above the blood in twenty-four hours. On the next day the broth has a rose-red color from hemolysis. On the fourth day the fluid is entirely red with a wreath of gas bubbles on the surface. The change in the blood is a production of methemoglobin. This blood destruction corresponds to the changes seen in large extravasations of blood (hematoceles) when they are infected with this organism. The methemoglobin may be absorbed and give the patient a definite icterus. In such patients Schottmüller has frequently observed methemoglobin in the blood.

In *serum sugar bouillon* an abundant turbidity develops in two to three days with a yellowish foam on the surface.

*Milk.*—Abundant development without change in appearance of the milk.

Best method for anaërobic fluid cultures is the absorption of oxygen by pyrogallic acid in tall glasses (Omeliansky's method), the surface of the media being covered with plastilin.

No hemolysis on blood agar plates.

Virulence not great for laboratory animals. Mice, guinea-pigs, and rabbits are resistant to large doses (2 to 5 c.c.), given subcutaneously or intraperitoneally.

Veillon and Zuber[3] mention an anaërobic staphylococcus as well as Mannu af Heurlin.[4] The latter may be identical with *Staphylococcus aërogenes*.

1. Schottmüller: Centralbl. f. Bakteriol. [etc.], 1912, lxiv, Loeffler's Festschrift, p. 270.
2. Schottmüller: München. med. Wchnschr., 1911, lviii, 1, 557; 787.
3. Veillon and Zuber: See Rist., Centralbl. f. Bakteriol. [etc.], 1901, xxx, 287.
4. Mannu af Heurlin: Bakt. Untersuchungen, etc. Helsingford, 1910, Verl. d. Akad. Buchhandlung.

### COSTA'S ANAËROBIC STREPTOCOCCUS

Found by Costa in a large abscess in the abdominal region and associated with Vincent's fusiform bacillus.

In the pus it appeared as a very small coccus in long chains like a streptococcus, but not staining by Gram, accompanied by Gram-positive, elongated, fusiform bacilli 4 to 8 $\mu$ long, with pointed ends. The bacillus was not cultivated, but a coccus in chains developed on media under anaërobic condition. In broth it produced a slight turbidity with a whitish sediment. Gelatin was slowly liquefied, milk not coagulated. Similar cocci and fusiform bacilli found in an abscess in the right tonsil.

Costa: Compt. rend. Soc. de biol., 1912, lxxii, 847.

### STAPHYLOCOCCUS ASACCHAROLYTICUS Distaso

Isolated from the intestinal contents by Distaso.

Morphologically a diplococcus, a short-chained streptococcus with 4 to 8 individuals or very large bunches. Individual cells are twice as large as those of the staphyloccus of Jungano.

Gram-positive.

Grows only as an anaërobe.

Colonies on *sugar agar* about as large as grains of sand; when separate, growing to the size of a pinhead. Microscopically they are transparent like a drop of liquid vaselin. Cultures emit a bad odor.

*Milk* acidified. No coagulation.

Grows in *gelatin* at 37° C. without peptonizing it and forming a precipitate like cotton wool at the bottom of the tube.

Growth in *white of egg* without attacking it and producing viscid zoöglœa.

No action on sugars.

Indol positive.

Regarded by the author as like the staphylococcus isolated by Jungano.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, 433.

### Anaërobic streptococcus of Marwedel and Wehrsig

Found in two cases of gas gangrene by Marwedel and Wehrsig.[1]

Gram-positive streptococci, often in lacy chains (6 to 10 diplococci), growing only as a strict anaërobe. According to Weinburg and Séguin it turns blood broth black and produces a putrid odor. Gives gas in glucose agar made from human placenta, the gas, however, coming from the proteins in the medium.

The organisms died out rapidly in cultures.

Virulent to guinea-pigs, 1 c.c. killing them in twelve hours by subcutaneous inoculation. At autopsy an extensive gelatinous hemorrhagic edema infiltrated with gas was found. Lesions not putrid. Pathogenic power lost rapidly.

Probably the same as *Streptococcus putridus* of Schottmüller.[2]

1. Marwedel and Wehrsig: München. med. Wchnschr., 1915, p. 1023.
2. Schottmüller: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1910, xxi, 450.

### Bloomfield's anaërobic coccus

Obtained by Bloomfield from lymphatic glands.

The colonies appeared in ascites agar tubes in two to five days, sharply limited to the subaërobic level, as discrete white pin-point disks. They were composed of Gram-positive cocci, about the size of the usual skin cocci, but differing from them in the early appearance of variations in size which was always striking by the time the colonies were large enough to be fished. Grown in subcultures only at the same level and not cultivated aërobically or anaërobically on the surface of plain, glucose, ascites, or blood agar, Loeffler's serum, in fluid media, or in simple dextrose agar stabs.

Bloomfield: Arch. Int. Med., 1915, xvi, 197.

### Adamson's anaërobic diplococcus

Cultivated by Adamson from septic wounds (18 out of 51 cases), not from the blood.

**Morphology.**—Moderate sized, Gram-positive diplococcus. The two cocci are unequal in size, the larger about like *Staphylococcus aureus*, the others smaller. Slightly flattened at point of union. United in chains in liquid media.

**Cultivated** only under anaërobic conditions.

*Agar colonies* small, regular, circular. The young colonies look like streptococcus colonies, while the old colonies are larger.

*Agar Slant.*—Transparent surface film.

*Glucose Agar.*—Growth more vigorous than on plain agar. Colonies larger, translucent, or even semiopaque. Isolated colonies five to six days old are 1 to 2 mm. in diameter, circular with semiopaque centers with 2 concentric rings, the inner more translucent. Marginal zone observed in older colonies.

*Glucose Agar Stab.*—No gas.

*Dorset's Egg Medium.*—Colonies small, transparent, superficial. No digestion, softening, or other change in medium.

*Egg Broth.*—No change.

*Inspissated Serum.*—Colonies small, superficial, whitish, semiopaque. No erosion of the surface or liquefaction.

*Broth.*—Not very abundant growth. Scanty deposit.

*Glucose Broth.*—Growth more abundant than in ordinary broth. No gas.

*No indol.*

*Gelatin.*—Not liquefied.

*Meat.*—No change.

*Milk.*—Strongly acid and clotted. No gas. No expression of whey (looks like *B. coli*). No peptonization.

Strongly acid, no gas with glucose, saccharose, lactose, and maltose. No change in mannite. Serum water media with sugars clotted.

**Quite Resistant.**—Agar cultures kept without sealing were alive after eighteen months. Not always killed in mixed cultures by heating to 80° C. one-half hour.

Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.

### Micrococcus minutissimus Oliver and Wherry

An obligate anaërobe isolated by Oliver and Wherry from the mixed flora in the aphthous ulcers of the gingival and buccal mucosa of a case of postpoliomyelitic paralysis.

**Morphology.**—Spherical non-motile cocci and diplococci, less than 0.5 $\mu$ in diameter.
**Stains** readily with the ordinary anilin dyes. Gram-negative.
**Grows** anaërobically by the pyrogallic acid method as discrete colonies on *sodium phosphate nutrient agar* containing pleuritic fluid. These colonies are opaque white and attain a diameter of 1 mm. When cultivated anaërobically on *sugar-free phosphate agar* (+ 0.8 to phenolphthalein) containing 1 per cent. dextrose, levulose, galactose, lactose, saccharose, maltose, or mannite and pleuritic fluid the growth is more luxuriant than on plain pleuritic fluid agar and bubbles of gas appear in the water of syneresis. When litmus is present in these solutions, after four days' growth, it is reduced. When the tubes are opened they turn deep blue. Gas also appears in sugar-free sodium phosphate broth; is non-absorbable by sodium hydroxid and is non-inflammable.
No aërobic growth.
Non-pathogenic to rabbits, guinea-pigs, and white mice.

Oliver and Wherry: J. Infect. Dis., 1921, xxviii, 341.

### Micrococcus of Hall

An anaërobic non-pathogenic micrococcus found by Hall in rhinitis.

Minute non-motile coccus, occurring singly, in pairs, and in small clumps. No chains observed. Gram-positive. Stains well with dilute carbolfuchsin, not so well with Loeffler's alkaline methylene-blue. Not acid-fast.
Growth somewhat slow on all media, better at 37° C. than at room temperature. Growth only anaërobic. Ferments glucose with acid formation. No gas. No indol produced. Delicate spreading film on *blood agar slants*, made up of minute bead-like colonies. *Deep glucose agar* shows fine colonies about 1 cm. below the surface. At the end of a week they are about a millimeter in size, dense, compact, and opaque.
No action on *milk*. No growth in *gelatin*.
Non-pathogenic for rabbits and guinea-pigs.

Hall: J. Infect. Dis., 1921, xxviii, 127.

### Thomson's anaërobic diplococcus from measles and scarlet fever

Minute Gram-negative diplococci, cultivated from the throats of measles and scarlet fever patients by Thomson on fluid testicular extract and on a mixture of soft agar and whole human blood.

The organisms appear as a minute diplococcus growing in pairs like the gonococcus and tending to be grouped in masses in liquid media. They measure about $\frac{1}{3}$ the diameter of the gonococcus or about 0.3 $\mu$. They are cultivated with great difficulty only under strictest anaërobic conditions. No growth under aërobic conditions. No growth on nutrient agar under anaërobic conditions. Grows with great delicacy only on media containing plasma, whole blood, hydrocele fluid, or testicular extract. On these media, colonies are exceedingly minute, almost transparent, like minute drops of moisture and tend to have a faintly yellowish tinge.
Large quantities of gas produced in testicular broth, testicular agar, plasma agar, and hydrocele agar to which no sugar is added. These culture media become highly acid. In Hiss's horse serum water the organisms ferment glucose, lactose, maltose, saccharose, dextrin, inulin, mannite, and glycerol, producing acid, but no gas.
Exceedingly soluble in weak alkali, resembling the gonococcus, meningococcus, and Pfeiffer's influenza bacillus.
The two strains, from measles and from scarlet fever, differ slightly. The scarlet fever strain formed large clumps in fluid media which on shaking appeared as flocculent masses. The measles strain did not tend to clump, but formed a fine uniform emulsion on shaking.
A rabbit fed on the measles strain developed after the second day a definite coryza, with running at the nose, sneezing, and discharge from the eyes. The diplococcus was found in the discharge from the nose. The animal was ill for two days, but recovered rapidly. The

organisms were not regarded definitely by Thomson as the cause of measles or scarlet fever. They could not be obtained from the blood during the commencement of the rash stage. They do not pass through a sound Berkefeld filter. They are present occasionally in the testicular mince cultures of sputum of normal individuals. They are, however, more abundant in the Gram-stained smears of the mucopus from the throats of measles and scarlet fever cases, while they are not evident in similar preparations from the throats of normal adults.

Thomson: J. Trop. M. and Hyg., 1923, xxvi, 1.

## FAMILY V. BACTERIACEÆ Cohn, emended Com.

Rod-shaped cells without endospores. Usually Gram-negative. Flagella when present peritrichic. Metabolism complex, amino-acids utilized, and generally carbohydrates.

### TRIBE I. CHROMOBACTEREÆ COM.

Water bacteria producing a red or violet pigment.

### GENUS 1. ERYTHROBACILLUS Fortineau

Small aërobic bacteria, producing a red or pink pigment, usually a lipochrome. Gram stain variable. It is possible that related yellow and orange chromogens should be included here as well.

**Organisms Producing Red Pigment.**—Reddish discolorations on foodstuffs have been known from early times and it is stated that as early as 332 B. C. Alexander was assisted in the conquest of Tyre by the "blood or bloody miracle." The appearance of reddish colorations on food, especially on bread, was usually ascribed to supernatural agencies and not infrequently this phenomenon led to merciless persecutions with the loss of hundreds of lives. The fact that these spots on bread were due to the growth of micro-organisms of some kind was first shown in the epidemic near Padua, Italy, in 1819 by Bizio[1] and by Sette.[2] These organisms were called *Serratia marcescens* by Bizio. Another outbreak was investigated in 1844 by the French Academy of Sciences.[3] In 1848 a phenomenon of the same sort occurred in Berlin and was thoroughly studied by Ehrenberg[4] who obtained an organism to which he gave the name *Monas prodigiosus*. Since that time organisms producing red pigment have been described in various parts of the world. Some of them are yeasts and it is probable that the bloody miracle may at times have been produced by members of this group. A few spirilla also produce a reddish pigment. The majority of organisms producing reddish growths however, are non-sporulating rods, although a few spore-bearers with this property have been described. Many of them conform more or less to the type established by Ehrenberg for his *Monas prodigiosus*. They are very common in the air and in dust, especially frequent in water. The subject has been very thoroughly studied by Mary Hefferan[5] from whose valuable paper many of the facts here given have been taken.

Rod-shaped bacteria producing reddish pigments may be classified according to the type of pigment produced. The first group or the "prodigiosus" group includes organisms producing a pure red pigment with varying quantities of orange and sometimes a small amount of blue which gives a violet or black tinge to the growth. The orange color diminishes as the cultures age and there is an addition of black. The pigmentation is variously described as "blood-red," "carmin," or "violet-red." The pigment is insoluble. The principal members of this group are as follows:

### Group I

Type species *Erythrobacillus prodigiosus* (Ehrenberg) Committee.
" *indicus* (Koch).
" *plymouthiensis* (Fischer).
" *kiliensis* (Breunig).

### Group II

The second group includes organisms producing a soluble red pigment. The first organism to be carefully studied was the bacillus found in the condition known as red milk in which the whole milk becomes red and is known as *Bacillus lactis erythrogenes*. The principal organisms in this group are:

*Erythrobacillus lactis erythrogenes* (Grotenfeld).
" *rutilescens* (Hefferan).
" *rubefaciens* (Zimmermann).
" *lacto-rubefaciens* (Gruber).

### Group III

The third group is a heterogeneous group embracing the organisms which produce insoluble pigments varying in color from orange and pink to red. They often grow slowly and according to Hefferan show much less diversity of biological properties than do the members of the prodigiosus group. For the present this group may be known as the "rubricus" group following Hefferan, and in it may be put a large number of red pigmented bacteria not definitely included in the previous two groups, including Hefferan's salmon-pink group.

The principal members are as follows:

*Erythrobacillus rubricus* (Hefferan).
" *ruber* (Zimmermann).
" *havaniensis* (Sternberg).

1. Bizio: Gazetta privilegiata di Venezia, Aug. 24.
2. Sette: Memoria storico-naturale cull' arossamento straordinario di aleune sostanze alimentose Venice.
3. French Academy of Sciences: J. de pharm., Jan., 1844.
4. Ehrenberg: Monatschr. über die zur Bekanntmachung geeigneten Verhandl. d. Kgl. preuss. Akad. der Wissenschaften Berlin.
5. Hefferan: A comparative and experimental study of bacilli producing red pigment. Jena, 1904.

#### Erythrobacillus prodigiosus (Ehrenberg)

First described accurately by Ehrenberg[1] as *Monas prodigiosus* and subsequently investigated by a number of observers. Description from Hefferan. Correct name apparently *Serratia marcescens* Bizio.

**Morphology.**—Small bacillus often appearing as a micrococcus under fairly low power of magnification (800 diameters). Usually about 0.5 $\mu$ broad and 0.5 to 1 $\mu$ long (Migula). It may occur in short chains of 3 to 6 elements. More short and coccus-like on alkaline media, more elongated and rod-like on acid media.

**Motility.**—Often difficult to make out and often very active especially in slightly acid media. Flagella peritrichic varying in number and length.

**Staining Reactions.**—Stains well by ordinary anilin dyes. Gram-negative.

**Cultural Characters.**—*Bacillus prodigiosus* grows well on all laboratory media.

*Agar Plate.*—Surface colonies in twenty-four hours are round, thin, flat; under low power slightly granular with thin transparent edges. Deep colonies are round or oval, under low-power opaque. The surface colonies become pigmented in two to three days, the characteristic reddish color appearing first in the center of the colony while the edges remain pale,

bluish, transparent. At the end of five days the surface colonies have a diameter of 3 to 7 mm. The agar itself does not become colored.

*Agar Slant.*—White, smooth, homogeneous moist layer in twenty-four hours. Often the growth is pigmented by this time, but frequently the characteristic pigment comes out in forty-eight to seventy-two hours. In three to four days the surface growth has a green fuchsin-like lustre. The agar does not become colored.

*Gelatin Plate.*—Colonies appear in twenty-four to forty-eight hours. Deep colonies are round, sharply contoured, gray and granular. Surface colonies are thin, whitish, flat and granular, round or irregular. The red pigment begins to appear in twenty-four to forty-eight hours, but liquefaction sets in rapidly and the pigment is almost immediately spread through the liquid gelatin. Often pigment production and liquefaction are synchronous and one gets only the pigmented liquid gelatin. No discrete pigmented colonies.

*Gelatin Stab.*—Rapid growth along the line of inoculation with deep funnel-shaped liquefaction in twenty four hours. The characteristic pigment is rapidly produced and sinks to the bottom as a red precipitate in the liquid gelatin. In forty-eight to seventy-two hours the gelatin is completely liquefied.

Fig. 67.—*Bacillus prodigiosus*, plain agar, twenty-four hours, stained with gentian-violet.

*Potato.*—An abundant growth usually in twenty-four hours, at first white, rapidly becoming red with a green metallic lustre. If the potato is moist the red pigment is spread over the entire potato. A slightly acid reaction favors the development which becomes more luxuriant.

*Blood-serum.*—Moderate growth at first, later becoming more abundant. The red pigmentation appears first in the center of the growth while the edges remain colorless. The medium is liquefied.

*Broth.*—Abundant turbidity in twelve to eighteen hours with later a ring of red color at the surface with a reddish-white sediment. The surface may have a reddish pellicle and the red pigment be diffused through the medium.

*Milk.*—Slow acidification and coagulation with a red color at the surface. According to Flügge and Migula the red pigment adheres to the fat globules. Later the clot shrinks and a clear water serum is squeezed out. Little or no peptonization.

*Fermentation Tubes:*

*Dextrose.*—In sugar-free broth neutralized by NaOH instead of $Na_2CO_3$ with $1\frac{1}{2}$ per cent. dextrose added, *Bacillus prodigiosus* produces gas slowly. The gas appears in about a week and slowly increases, the total amount being 34.5 per cent. The gas is all absorbed by NaOH. The broth becomes acid in reaction. No gas in lactose, saccharose, or in asparigin-sulphate-phosphate with 2 per cent. dextrose.

*Nitrates* are reduced to nitrites by *Bacillus prodigiosus* with sugar-free broth and 1 per cent. $KNO_3$ in the fermentation tube; gas bubbles appear in about a week.

**Odor.**—*Prodigiosus* cultures give off a strong odor of trimethylamine. This is absent in non-protein media.

1. Ehrenberg: Monatschr. über die zur Bekanntmachung geeigneten Verhandl. d. Kgl. preuss. Akad. der Wissenschaften Berlin.
2. Hefferan: A comparative and experimental study of bacilli producing red pigment. Jena, 1904.

**Varieties of Bacillus Prodigiosus.**—Several varieties of *Bacillus prodigiosus* were found by Hefferan. One of these fermented saccharose as well as dextrose. Another fermented dextrose, saccharose, and lactose. Another produced a viscid growth in ordinary media giving off long threads when touched with the platinum needle, and produced no gas in sugar media. Other strains produced pigments of a slightly different color from the typical "prodigiosus."

### ERYTHROBACILLUS INDICUS (Koch)

This organism was found by Koch[1] in the stomach contents of an Indian ape and described by him as *Bacillus ruber indicus*. Kruse[2] states that it was also found by Pasquale in Massua. The original *Bacillus indicus* produced pigment at 37° C. and was toxic to rabbits and guinea-pigs. The strain in the Johns Hopkins Pathological Laboratory is a descendant of the original Koch culture. It gives the following reactions:

Fig. 68.—*Bacillus indicus*, plain agar, twenty-four hours, stained with gentian-violet.

**Morphology.**—Organisms from plain agar twenty-four hours old when stained by gentian-violet measure about 0.4 $\mu$ in breadth and 1 to 2 $\mu$ in length. They are somewhat thicker on dextrose agar and much longer, often 4 to 6 $\mu$.

*Staining Reactions.*—Stains easily with the usual dyes. Gram-negative.

*Motility* active, whirling, and progressive.

*Cultivated* readily on the usual media.

**Cultural Reactions.**—*Plain Agar Plates.*—Colonies $\frac{1}{2}$ to 1 mm. in diameter in twenty-four hours, delicate and white. Under low power opaque, round, regular, granular, margins entire. In forty-eight hours the colonies are larger and distinctly reddish. Deep colonies small ovals and disks. After eight to ten days the colonies are much larger, usually 6 to 8 mm. in

breadth, dark red in color (like methemoglobin) in the center, with thin white margins. Older colonies may show a peculiar fine wrinkling.

*Plain Agar Slant.*—Moderately thick whitish growth in twenty-four hours. In two to three days the growth is more abundant, pale rose-colored, especially at the bottom. In cultures eight to ten days old the growth is heavy and fairly red.

*Plain Agar Stab.*—Pale line growth, abundant surface expansion, slightly reddish in color.

*Dextrose Agar Plates.*—Round, regular, surface colonies with entire, slightly irregular margins. Deep colonies ovals and disks. Bottom colonies colorless. Agar acid, no gas. In old plates the surface colonies are rose-red or brick-red. The color is not so dark as that of *Bacillus prodigiosus*. The agar turns alkaline.

*Dextrose Agar Slant.*—Abundant whitish expansion, rose-red in color. Reaction at first acid then alkaline.

*Broth.*—Turbidity and sediment. No scum. In old cultures the sediment is distinctly reddish.

*Peptone.*—Turbidity, sediment and rose-red scum.

*Litmus Milk.*—Acidity and coagulation. Partial digestion to a yellow fluid in old cultures.

*Potato.*—Abundant rose-red, almost blood-red expansion.

*Blood-serum.*—Abundant, pale red growth with some softening of the serum, but no definite liquefaction.

*Gelatin Plates.*—Colonies appear in forty-eight hours, small and discrete, brick-red in color. The gelatin is rapidly liquefied and assumes a reddish line.

*Gelatin Stab.*—Abundant line and surface growth with a cup-shaped liquefaction in two to three days. The liquefaction is soon complete, the liquefied gelatin being brick-red in color. In old cultures a red film or scum may form on the surface.

*Carbohydrates.*—Acid, no gas from dextrose, saccharose, and lactose. According to Hefferan this organism produced gas, all $CO_2$, from dextrose and saccharose.

1. Koch: Berichte über die Reise zur Erforschung der Cholera. 1884.
2. Kruse: *In:* Die Mikroorganismen (Flügge). Leipzig. 3 Aufl. 1896, p. 302.

### ERYTHROBACILLUS PLYMOUTHIENSIS (Fischer)

Originally obtained by Fischer from water and described by him as *Bacillus ruber plymouthiensis*. This organism differs from standard "prodigiosus" cultures by its slower liquefaction of gelatin, its vigorous production of gas in dextrose, saccharose, and lactose. Of this gas 70 to 78 per cent. is $CO_2$. Gas is also formed in a standard asparigin sugar solution of which 42 per cent. is $CO_2$. Cultures have a strong fecal odor.

Fischer: Ztschr. f. Hyg., 1887, ii, 74.

### ERYTHROBACILLUS KILIENSIS (Breunig)

This organism was obtained from water by Breunig[1] and described as *Bacillus ruber balticus* by Kruse.[2] Investigated by Laurent, who calls it "Bacille rouge de Kiel." The following description is taken from Hefferan.

**Morphology.**—Small rods on young potato culture measuring 0.6 to 0.8 $\mu$ in diameter by 2.5 to 5 $\mu$ in length. In old potato cultures the organisms may be 8 $\mu$ in length. Motility in very young cultures on potato at 37° C. (four hours) and at room temperature in twenty-four hours.

**Cultural Characters.**—Rapid liquefaction of gelatin with a thick orange-red surface pellicle. Color on potato at first red violet, later darker like iron rust. Often with a green lustre. Broth clouded with a thick orange-red pellicle and red sediment. Gas in dextrose saccharose, and lactose. Of this only a small part, 20 to 28 per cent., is $CO_2$. In standard asparigin solution with 1 per cent. dextrose, gas was produced to 14.3 per cent. of the tube length, none of it $CO_2$. Pigment production at 37° C. violet-red, not like the orange-red seen at room temperature. Nitrates reduced to nitrogen gas. Odor not characteristic.

1. Breunig: Bakteriologische Untersuchungen des Trinkwassers der Stadt Kiel. Diss. Kiel, 1888.
2. Kruse: *In:* Die Mikroorganismen (Flügge), 1896, p. 303.
3. Laurent: Ann. de l'Inst. Pasteur, 1890, iv, 465.

### ERYTHROBACILLUS LACTIS ERYTHROGENES (Grotenfeld)

Found by Hueppe in red milk in which the whole fluid is turned red. Described in 1889 by Grotenfeld as *Bacillus lactis erythrogenes*. Obtained also by Hefferan from Mississippi River water. The following description corresponds to Grotenfeld's original statements and to the organisms isolated by Hefferan.

Small rod 1 to 1.4 $\mu$ long, non-motile. Gelatin colonies small, round, at first gray-white, later yellow. Gelatin liquefied, taking on a rose tint outside the colonies. Gelatin stab liquefied slowly from the top. The liquid gelatin is red with a yellow sediment; after ten to twelve days the solid gelatin below being a weak rose color. Agar growth, moist luxuriant yellow, the agar becoming rose-colored and even wine-red. Yellow growth on potato at 37° C., becoming deep golden in six to eight days. The potato often shows a slight pink tinge and the potato growth becomes dark and discolored.

Broth cloudy and yellow. Milk shows a slow precipitation of casein with a clear serum which becomes blood-red. Reaction alkaline. No liquefaction of blood-serum. No gas from sugar. No anaërobic growth. Pigment production at 37° C. Filtrate reduced to nitrites.

Non-pathogenic.

Cultures have a characteristic sweet odor.

Grotenfeld: Fortschr. der Med., 1889, vii, 41.

### ERYTHROBACILLUS RUTILESCENS (Hefferan)

Isolated from the Mississippi River by Hefferan.

Actively motile bacillus.

White, non-characteristic colonies on gelatin which is rapidly liquefied. Gelatin stab also rapidly liquefied with white cloudy sediment and white pellicle. Later the liquid gelatin takes on a beautiful clear rose color, and the floating pieces of sediment take on a pink tinge. Agar colonies often spreading without pigment. Luxuriant, smooth, moist, white growth on agar slant. Luxuriant white spreading growth on potato. Broth shows marked turbidity with white sediment and thin pellicle. Litmus milk slightly acid, coagulated in three days at 22° C. Coagulation in forty-eight hours at 37° C. with partial peptonization. No gas production. Nitrates reduced to nitrites. Facultative anaërobe. Growth at 22° C. and at 37° C.

### ERYTHROBACILLUS RUBEFACIENS (Zimmermann)

Isolated from Chemnitz water supply by Zimmermann.

Thin, actively motile bacillus 1 to 1.6 $\mu$ long, often in short chains of 2 to 3 elements.

Gelatin colonies minute white dots in forty-eight hours. Under low power granular, pearly, thinner at the edges which are well defined. Gelatin stab yellowish growth along line of inoculation, giving a reddish tinge to the medium. No liquefaction. Agar colonies small, white, with fragmented edges. Agar slant white, smooth, shining, somewhat luxuriant growth. In old cultures the agar takes on a distinct, clear, wine-red, transparent color. Heavy cream-white color on potato, later yellow-brown. Broth turbid with a white surface pellicle, slightly reddish after six to eight weeks. Litmus milk decolorized, acid coagulates only on boiling. No gas from carbohydrates. Nitrates reduced to nitrites. Facultative anaërobe. No growth at 37° C.

Note: The pigment produced by *Bacillus rubefaciens* is diffusible through gelatin and agar.

Zimmermann: Die Bakterien unserer Trink- und Nutzwässer. Chemnitz, 1890, p. 26.

### ERYTHROBACILLUS LACTORUBEFACIENS Gruber

Found originally by Gruber in red milk.

Actively motile.

Colonies like *Bacillus coli*. White, spreading growth on agar slant and potato. Growth in depths of gelatin stab sometimes arborescent. Broth turbid with pellicle and sediment. Milk becomes rose-colored, very slimy. Litmus milk slightly acid. No coagulation at room temperature. No growth at 37° C.

Gruber: Centralbl. f. Bakteriol. [etc.], 1902, viii, 457.

### ERYTHROBACILLUS RUBRICUS (Hefferan)

Isolated from the Mississippi and Missouri Rivers by Hefferan. The organisms differ from *Bacillus prodigiosus* in their slow growth and slow pigment production. Pigment at first pink, characteristic brilliant red after some weeks.

Small, slender, non-motile bacillus. Gelatin and agar colonies small, round; under low power finely granular with yellow-orange color deepening to red. Liquefaction of gelatin very slight when first isolated. Later lost. Bright pigment on dry agar slant in ten days. On moist agar, growth remains colorless or slight pink, the red color coming out very slowly. Little or no growth on potato. Turbidity in broth with pink pellicle. Alkaline reaction in litmus milk. No gas from sugars. No reduction of nitrates. Grows at 37° C., better at room temperature.

### ERYTHROBACILLUS RUBER Zimmermann

Cultivated from the Chemnitz water supply by Zimmermann and regarded as almost identical with the red bacillus described by Eisenberg. Called *Bacillus pseudoruber* by Migula because of the previous description of a red organism by Frank under the name *Bacillus ruber*.

Krals culture sent to Mary Hefferan showed an actively motile bacillus. No liquefaction of gelatin. No gas from sugars. Slow, clear, red growth on potato limited to line of inoculation. Broth showed a thin, pink surface pellicle from which floating cobwebby streamers dipped down into the clear fluid. Occasionally pin-point pink colonies were found attached to the wall of the tube. Litmus milk alkaline. No anaërobic development. No growth at 37° C. No liquefaction of blood-serum. No reduction of nitrates.

Zimmermann: Die Bakterien unserer Trink- und Nutzwässer. Chemnitz, 1890, p. 24.

### ERYTHROBACILLUS HAVANIENSIS (Sternberg)

Isolated and described by Sternberg as a short oval bacillus, usually in pairs, 0.4 to 0.5 $\mu$ in diameter.

An aërobic, non-liquefying, slowly motile organism growing slowly at room temperature. Spherical, translucent colonies on gelatin plates, blood-red in color. Scanty, colorless growth in the depths of gelatin stab with an opaque carmin layer on the surface. Slow growth on agar with wavy outlines glistening red. Seldom grows on acid potato, may grow on old dry potato. Pigment production only in presence of free oxygen. (No gas from sugars. No pigment at 37° C. Hefferan.) According to Hefferan a strain marked *B. havaniensis* in the bacteriological laboratory, University of Chicago, closely resembled *Bacillus ruber* of Zimmermann or her *Bacillus rubricus*.

Not *Bacillus hananiensis* of Kruse and Migula, the organism originally described by Sternberg as *Bacillus havaniensis liquefaciens*.

Sternberg: Manual of Bacteriology. 1892, p. 718.

## GENUS 2. CHROMOBACTERIUM Bergonzoni

Aërobic bacteria, producing a violet chromoparous pigment, soluble in alcohol, but not in chloroform. Motility and Gram reaction variable.

Organisms producing violet pigments were isolated early in bacteriology when examinations were made of water and air. The older descriptions are incomplete and of historical importance only. According to Bampton there are 2 main types of liquefying violet-producing organisms which are more or less homogeneous by immunological reactions: *Bacillus violaceum* of which he studied 18 strains and *Bacillus membranaceus amethystinus* of which he studied 4 strains. The most important organisms of this group are the following:

Type species *Chromobacterium violaceum* (Bergonzoni).
" *violaceum lutetiense* (Kruse).
" *violaceum laurentium* (Jordan).
" *violaceum Manilæ* (Woolley).
" *nubile* (Frankland).
" *membranaceum amethystinum* I (Bampton).
" *membranaceum amethystinum* II (Bampton).
" *membranaceum amethystinum* III (Bampton).
" *membranaceum amethystinum* IV (Bampton).
" *janthinum* (Zopf).
" *cæruleum* (Smith).

### CHROMOBACTERIUM VIOLACEUM (Bergonzoni)

Originally reported by Bergonzoni[1] as *Bacillus violaceus*. Present description from Bampton[2] who isolated the organism from the Hamburg water supply.

**Morphology.**—Twenty-four-hour agar culture at 22° C. Thick rods with rounded ends, 2.1 to 4.2 μ long, less than .05 μ thick, in young cultures forming short threads, in old cultures longer threads.

**Motility.**—Rotating, vibrating, serpentine, like that of the typhoid bacillus. Flagella 3 to 4 peritrichic, 1 to 2 polar.

**Staining Reactions.**—Gram-negative. The staining is usually not homogeneous. In many instances Gram's stain brings out more deeply stained granules in the bacterial substance. These also appear after longer application of Neisser's stain than is usually employed for diphtheria. The bacillus is thus very much like the diphtheria bacillus in morphology and cannot be differentiated from it. The blue-stained bodies or granules are usually terminal, sometimes central, usually not as wide as the organism. Sometimes they are more voluminous, however, than the protoplasm of the bacteria which takes the brown stain. Not acid-fast. No spores.

Note: Stained with carbol methyl violet, treated with iodin in potassium iodid solution, decolorized with acetone alcohol, counterstained with carbolfuchsin 1 to 9.

**Cultivated** without difficulty; growth fairly rapid, best at room temperature. Good growth also in the ice-box (+ 6° C.) with good pigment formation. No growth at 37° C.

*Gelatin Plates* (alkalinity 0.054–0.058).—In twenty-four hours colonies appear as delicate bluish-white points, reaching a diameter of 0.5 mm. in two to four days. The superficial colonies sink into the gelatin and have an appearance of air bubbles with a deep violet center. In the periphery the color gradually becomes weaker, turns to a brownish-yellow and shows larger granules than the center, which is finely granular. Some colonies are colorless in the middle with deep violet, finely radiating edges. Deep colonies two to four days old have a diameter of 1.3 to 2.6 mm., are whitish-yellow, coarsely granular. The edges are irregular and sometimes provided with short processes. Deep colonies when older have narrow violet rings in the colorless zone of liquefaction, which begins in the upper part. With a magnification of about eighty times the superficial colonies have a deep violet color and an irregular, lobulated peripheral zone. The deep colonies show brownish-yellow masses in the center with pale violet peripheral zones which take on again a brownish-yellow color toward the periphery.

*Gelatin Stab.*—Slight development in twenty-four hours; some superficial liquefaction. Finely granular masses develop in the stab after three to four days and a funnel-shaped liquefaction with violet granular masses at the lower part of the stab and unstained above. After a week the zone of liquefaction has spread in the upper third and looks like a retort. On the surface of the liquefaction there is a violet film and in the stab fine, usually colorless floccules. After two weeks complete liquefaction occurs with a violet film on the surface and a violet sediment. Later the violet pigment diffuses from the surface into the depths of the medium.

*Agar Plates* (extract agar, alkalinity, .05–.06).—Growth slower and less extensive than on gelatin. Colonies round, colorless, or pale violet at first, with moist, slightly convex surfaces. Three-day colonies have a diameter of about 1.0 μ and consist microscopically of 3 zones. The central zone is brownish and coarsely granular, the middle zone violet with a flocculent appearance, while the outer zone is narrower than the others, yellowish, homogeneous. The three zones are not sharply separated, but fuse into each other. Other colonies

are violet throughout. Colonies six days old are round, or slightly convex, have a diameter up to the size of lentils, and are deep violet, moist, and glistening.

*Agar Slant.*—In twenty-four hours slight development along the line of inoculation; moist, pale violet. Older cultures show slightly toothed edges. The color turns to dark violet or black violet.

On agar of natural acidity pigment production is increased. The growth is moist, glistening, and slightly granular.

*Loeffler's Blood-serum.*—Growth moist and more abundant than on agar. Pigment definitely blue. Along the line of inoculation the growth is dark with a bluish-white periphery. The pigment diffuses into the media and takes on a blue-violet color. After a week the medium is completely blue, partially liquefied. The liquefied portion has a violet color.

*Potato* (Globig's medium).—No growth.

*Milk.*—Slight pigment production, chiefly in the superficial zone. No acid production. No clotting.

*Bouillon.*—Slight turbidity in two to three days with the formation of a whitish sediment. In older cultures the sediment is more marked and has a violet color. No appreciable superficial film.

*Peptone Solution 1 per cent.*—Turbidity, no film. No indol.

*Grape-sugar Bouillon.*—Growth like that in ordinary bouillon. No gas.

*Neutral Red Agar Slab.*—Slow development with formation of a violet membrane on the surface. No fluorescence.

*Litmus Milk-whey.*—Increase of color after fourteen days.

*Litmus Sugar Nutrose Media* of Hetsch.—*Grape-sugar*, turbidity, reddening in two days, clotting in a week; no gas. *Maltose*, slight reddening after two days. Turbidity, no clot. *Mannite*, slight turbidity, no reddening, no clotting. *Saccharose*, very slight turbidity, no reddening, no clotting. *Lactose*, slight turbidity, no reddening, no clotting. No *hemolysis* with sheep and calf blood.

Slight anaërobic development. The anaërobic colonies form no pigment and look like the deep colonies on gelatin plates.

Slight development on casein agar and casein gelatin. Little pigment production. The gelatin is barely liquefied by the growth, but consumed.

No indol formation. No $H_2S$.

**Remarks.**—Bampton has studied 17 other strains of *Bacillus violaceus* from different sources and found that they differed but little from his typical Hamburg strain. By serological reactions they are evidently a homogeneous group.

1. Bergonzoni: Societa d. Naturalisti in Modena, 1881, 2, xiv, 149.
2. Bampton: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1913, lxxi, 129.

### CHROMOBACTERIUM VIOLACEUM LUTETIENSE (Kruse)

Originally described by Macé[1] and named by Kruse.[2]

**Morphology.**—Small rods, 0.5 to 0.7 $\mu$ wide and 1.5 to 2 $\mu$ long. No spores.

**Cultivated** without difficulty on the usual media.

*Gelatin.*—Rapid liquefaction with a delicate whitish-gray pellicle which falls into the liquid gelatin and remains attached to the walls of the tube. After a month or two at room temperature the borders show a delicate violet color in the parts which emerge from the liquid. The culture develops a cheesy odor.

*Agar.*—Growth more abundant. At the end of two to three days a small white expansion appears at the point of inoculation. This increases in size and after a week becomes a thick pellicle marked by circular hyaline striations. In two weeks the area of growth is 2 cm. in diameter with borders of uniform thickness. The violet color appears along the concentric striations from the periphery to the center. At the end of six weeks the growth is 3.5 to 4 cm. in diameter and is entirely violet. The color is very dark, exactly like a concentrated solution of methyl violet. The surface color deepens and becomes a black-violet, while the pellicle wrinkles.

No growth on *potato*.

The pigment is insoluble in water and alcohol, and needs air for its production. The odor of the cultures is first cheesy, later acid like that of butyric acid.

1. Macé: Ann. d'hyg. publ. et de méd. lég., 1887, xvii, 354.
2. Kruse: *In:* Handb. d. path. Mikroorg. (Flügge). 1896, ii, 311.

### CHROMOBACTERIUM VIOLACEUM LAURENTIUM (Jordan)

Found by Jordan in the sewage at Lawrence, Mass.

**Morphology.**—Bacilli with rounded ends from 3 to 3.6 $\mu$ long and 0.7 $\mu$ broad. Often in pairs, sometimes in chains of 4 to 5 elements.

**Motility** active.

**Cultural Characters.**—Grows on the ordinary laboratory media, best at the room temperature.

*Agar Slant.*—An abundant development at first dark violet, later jet-black.

*Gelatin Plates.*—At the end of two days deep colonies, small, spherical, coarsely granular. Usually with dark centers and radiating margins. When the colonies come to the surface they form thin, irregular expansions. The gelatin is rapidly liquefied in the vicinity of the colonies. The centers are violet-colored spots about which are zones of slightly clouded liquid gelatin. Liquefaction progresses rapidly, but the central mass does not materially increase in size.

*Gelatin Stab.*—Rapid liquefaction along line of inoculation with a cloudy violet-colored liquid gelatin and an abundant dark violet precipitate at the bottom of the tube.

*Potato.*—An abundant dark violet growth spreading over the entire piece of potato. The growth soon becomes jet-black except at the edge.

*Milk.*—Rapid development causing early coagulation and an acid reaction. The milk acquires a deep blue-violet color.

*Broth.*—Growth, but no violet color. When nitrates are added the growth is more luxuriant and a rich violet color is produced.

Nitrates reduced to nitrites, rather slowly.

Jordan: Report Mass. State Board of Health, 1890.

### CHROMOBACTERIUM VIOLACEUM MANILÆ (Woolley)

Isolated by Jobling and by Woolley from caraboas dying acutely and found in large numbers in the lymph-glands and lungs.

**Morphology and Staining Reactions.**—Short rods measuring approximately 0.5 by 1 to 1.5 $\mu$. Occasionally rods are even longer. It stains with the usual anilin dyes, best perhaps with carbol thionin, or with carbolfuchsin or gentian-violet diluted five times with water. Gram-negative. Not acid-fast. When well stained it appears as a short, thin bacillus, very frequently slightly bent, with rounded ends. It does not as a rule stain uniformly and may show one or more clear spaces which are not tinted and which appear remarkably like spores. These clear spaces can not be stained by the usual methods used in coloring spores. The organisms are generally single, sometimes in pairs, occasionally arranged in short chains of 3 or 4 individuals. They are motile, usually sluggishly turning and twisting, but frequently single rods may be seen to cross the field of the microscope with a more rapid wriggling motion. Each organism possesses one polar flagellum. In rare instances two flagella may be distinguished springing from the same pole of the bacillus.

**Cultural Characters.**—On *agar plates* within twenty-four hours at 37° C. colonies appear as small, round, violet-gray dots. These slowly enlarge and become deeper in color. The maximum depth of color is attained in forty-eight to seventy-two hours. As the colonies grow, their margins become slightly irregular and with a rather indefinite concentric arrangement of layers, which are somewhat thicker toward their peripheries, with the result that the centers of the colonies have a less intense blue color than the edges. These masses of organisms are slightly tenacious, but after removal from the medium are readily dissociated in water.

On *agar slants* the growth is similar to that on plates, extension being slow, but rather even, resulting in a very slight crenation. The water of condensation is clouded and bluish, with a blue sediment.

In *agar stab* cultures the growth is scant, and no pigment is formed below the surface.

*Gelatin* is liquefied slowly at the temperature of the ice-box, the growth forming a funnel-shaped area with a cup-shaped upper portion. The sediment is bluish.

*Bouillon* is diffusely clouded. A delicate pellicle is formed which upon 1 per cent. alkaline (to phenolphthalein) material is but slightly pigmented, but which is better developed and bluer on a 1 per cent. acid liquid. The sediment in 1 per cent. acid bouillon is pale blue violet and rather viscid.

Dunham's *peptone* solution is also clouded, and a thin pigmented pellicle is produced. A sediment is deposited in this medium which resembles that in broth, but in which more pigment is present. The whole medium becomes diffusely bluish within forty-eight hours. On *potato*, the growth extends over the whole surface of the medium and to the water of condensation. The superficial layers of all the solid media are stained by the soluble blue pigment. The pigment production is luxuriant.

In *sugar-containing media*—glucose and lactose—no gas is formed.

The reaction of *milk*, after seven days, is slightly acid, but there is no coagulation. The casein is peptonized and the upper third of the tube becomes almost clear and faintly violet.

The organism thrives better and produces pigment more freely at 37° C. than at lower temperatures. It does not grow at all well and does not produce any pigment below the surface of a solid medium. It is almost an obligate aërobe.

It is killed by boiling for one minute, by a temperature of 67° C. after an exposure of five minutes, and by a temperature of 57° C. after one hour. It does not form spores.

The pigment is soluble in alcohol, giving a deep rich violet solution. It is slightly soluble in water, barely dissolved in ether, and insoluble in chloroform.

**Pathogenic Action.**—Virulent to guinea-pigs by subcutaneous inoculation in doses of 1 c.c. of a forty-eight-hour culture. Death occurred in five days with a large necrotic area at the site of inoculation, hemorrhagic infarcts in the lungs, an enlarged soft spleen mottled with miliary gray spots, and similar spots in the liver. The organism was recovered from the blood and organs. Virulent to rabbits by subcutaneous inoculation in thirty-six hours, the animal showing at autopsy a wide-spread hemorrhagic lesion at the site of inoculation with miliary abscesses of the liver. Subcutaneous inoculation produced a local abscess in the cat, the abscess discharging, the skin sloughing and leaving an ulcer which healed. Similar lesions produced in dogs and calves. Intraperitoneal and intravenous inoculation also fatal to rabbits within a few hours.

Monkeys fed agar cultures showed no ill effects.

Animals (monkeys, guinea-pigs) given repeated injections develop agglutinins for the organism in their blood, running up to a dilution of 1 : 1000. No bactericidal action demonstrated.

Cultures filtered through Pasteur-Chamberland bougie F contained no soluble toxin.

Woolley: Publication No. 15, 1904, Bureau of Government Laboratories Department of the Interior, Manila.

### CHROMOBACTERIUM NUBILE (Grace and Percy Frankland)

Cultivated by the Franklands from filtered London water.

**Morphology.**—Thin, slender bacilli about 0.3 $\mu$ broad and 3 $\mu$ long. In preparations from gelatin cultures the organisms usually are single or in short chains, in broth the chains are longer and often wound in spirals. On potato the bacilli are curved, occasionally comma-shaped and spirals. No spores.

Hanging drops show long wavy threads. The individual organisms show active whirling movements without change of place.

**Cultural Characters.**—*Colonies* on *gelatin plates* are very characteristic. After forty-eight hours they appear as small turbid flecks not sharply differentiated from the surrounding gelatin. Under low power they appear as delicate colonies. A day later these flecks appear as thick, felty meshworks of bacilli. Occasionally the colonies show a thicker central area leading off to the surrounding network of threads. Rapid liquefaction, the individual colonies coalescing and destroying the whole plate.

*Gelatin Stab.*—Surface, funnel-shaped liquefaction which does not advance rapidly. The liquid gelatin is turbid, with a pale yellow sediment at the bottom of the funnel of liquefaction. About the line of inoculation, serving as an axis, a series of horizontal flat rings appear. These consist of isolated delicate clouds. The cultures look somewhat like those of mouse septicemia. Eventually the entire tube is liquefied.

*Agar Slant.*—Thin, opalescent bluish-white expansion with fringed edges which assume a violet fluorescence.

*Bouillon.*—Turbidity, dirty-white sediment, thin surface pellicle, which sinks on shaking.

*Potato.*—Delicate, pale yellow, almost invisible growth covering whole surface.

Nitrates reduced somewhat to nitrites and moderate amount of ammonia produced in solutions of calcium nitrate, grape-sugar and peptone.

Frankland, G., and P.: Ztschr. f. Hyg., 1889, vi, 373.

### CHROMOBACTERIUM MEMBRANACEUM AMETHYSTINUM I (Bampton)

Isolated by Bampton from the Hamburg water supply.

**Morphology.**—On twenty-four-hour agar culture rods 1.4 to 3.5 $\mu$ long, with rounded ends, somewhat thicker in the middle than on the ends, in short threads.

**Motility** active, more rapid than that of *Bacillus violaceus*, rotating, vibrating. Flagella 4 to 5 peritrichic, 1 to 2 polar.

**Staining Reactions.**—Stains homogeneously. Gram-negative. By Neisser's stain, applied longer than with the diphtheria bacillus, the cytoplasm is colored brown and contains small blue granules, smaller than those in the diphtheria bacillus, usually terminal, occasionally central, less abundant than with *Bacillus violaceus*. Not acid-fast. No spores.

**Cultural Characters.**—*Growth* slow, best at room temperature, lacking at 37° C., good in the ice-box.

*Gelatin Plates* (alkalinity 0.054 to 0.058 per cent.).—After twenty-four hours very small bluish-white points. After three days the superficial colonies have taken on an appearance of slightly convex, bluish-white, slightly granular membranes with irregular edges. Deep colonies much smaller, roundish, bluish-white. By a magnification of about 85 the superficial colonies appear gray-white, with irregular edges, with a small, light yellow, central zone, which passes outward into a broad, irregular, felt-like granular zone. Other superficial colonies are not membranous, but smaller, round, convex, moist, bluish-white. After a week the membranous colonies are 1 to 5 mm. in size. The irregular jagged edges and the peripheral zone in some colonies pale violet. Centers opaque, whitish-yellow. No liquefaction.

*Gelatin Stab.*—Up to forty-eight hours whitish growth on the surface in the neighborhood of the point of inoculation. No liquefaction. After a week the growth spreads to the edge of the tube and takes on a pale violet color. Later a moderate funnel-shaped liquefaction develops. The membrane creeps up the sides of the glass, is folded and pale violet in color. It is so adherent to the liquefied gelatin that it does not fall when the tube is turned over. Later the membrane takes on a deeper violet color and on the bottom of the liquefied gelatin, which has a brownish tone, a violet sediment collects.

*Agar Plates* (extract agar 0.05 to 0.08).—Growth slower than on gelatin. Colonies three days old are small, roundish, convex, yellowish-white in color with moist surfaces. With a magnification of 85 diameters the center appears yellowish-brown and finely granular, with a peripheral radiating zone. After eight days the colonies are of various sizes, the smaller white or very pale violet, the larger violet and roundish with irregular edges. They consist of 3 zones, the central zones of pale violet being enclosed in a middle band of deep violet from which fine violet streaks radiate into an outer more yellowish zone.

*Agar Slant.*—After twenty-four hours fairly abundant membranous-like growth, whitish in color. After forty-eight hours the peripheral zones are more abundantly developed. After a week the culture is a pale yellow with a trace of violet in the lower portions. Growth finely granular. On agar of its natural acidity the growth is moist, slightly raised, violet. Edges are deeper violet than the middle, later the color is uniformly deep.

*Loeffler's Blood-serum.*—Growth more rapid than on agar, after twenty-four hours very pale violet color with raised edges, toothed. After three days slight liquefaction and very slight diffusion of the pigment into the medium.

*Potato* (Globig's medium).—Abundant, membranous, raised, moist growth in twenty-four hours, brown or very pale violet in color with granular surface.

*Milk.*—No clotting. After two to three weeks some clearing (digestion). Violet membrane on the surface. Reaction slightly alkaline.

*Litmus milk* turns blue with membrane formation. It is later decolorized.

*Bouillon.*—After a few days a turbidity with a whitish sediment and a thin very pale violet membrane on the surface. Later the membrane is thicker, crumbly and sinks to the bottom.

*Grape-sugar Broth.*—Growth the same. No indol.

*Peptone Water.*—Growth the same. No indol.

*Neutral Red Agar Stab.*—Slow growth with the formation of a membrane, at first white, then brown on the surface, and a decolorization of the medium.

*Litmus Sugar Media* (By Hetch's method).—*Grape-sugar*, no gas. At first reddening, after a week turbidity with whitish violet sediment, decolorization of the medium, pale violet membrane on the surface. *Maltose*, slight reddening of medium with a turbidity and whitish sediment. Later decolorization, pale violet membrane formation. *Mannite*, very slight reddening and slight turbidity, later appearance like that in mannite, membrane bluish-white. *Lactose*, at first no change in the medium, later a slight turbidity with white sediment. In older cultures pale bluish color with membrane formation.

No *hemolytic* action on sheep or calf blood. Slight anaërobic growth without pigmentation.

*Casein Agar and Gelatin.*—Slow growth, little pigmentation.

No $H_2S$.

Bampton: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1913, lxxi, 129.

### CHROMOBACTERIUM MEMBRANACEUM AMETHYSTINUM II (Bampton)

Described by Bampton from the Hamburg water supply.

**Cultural Characters.**—*Gelatin surface colonies*, six days old, pale violet, concave, sunken to the bottom of the Petri dishes, the middle appearing as a small violet point surrounded by a colorless zone, with a violet outer zone.

*Agar Stab.*—Growth at first gray-white, later violet. The pigmentation of the irregular edges is deeper than that of the middle.

*Loeffler's Blood-serum.*—No liquefaction. Whitish-violet growth.

### CHROMOBACTERIUM MEMBRANACEUM AMETHYSTINUM III (Bampton)

Described by Bampton from the Hamburg water supply.

Superficial *gelatin* colonies fourteen days old, violet, folded, membranous-like, irregular edges, roundish, concave, sunken to the bottom of the Petri dishes. Gelatin used up by the growth of the membrane. When the whitish-yellow, deep colonies reach the surface, the centers take on a pale violet color, the middle zone a dark violet color. Gelatin partly liquefied.

*Agar cultures* three days old have a slightly convex, moist surface and consist of only a single zone.

*Loeffler's Serum.*—Very moist, bluish-white growth. No pigmentation of the medium.

*Potato.*—Yellow-brown membrane. Granular, somewhat dry.

### CHROMOBACTERIUM MEMBRANACEUM AMETHYSTINUM IV (Bampton)

Described by Bampton from the Hamburg water supply.

Stained by Neisser, polar granules more abundant than in No. I.

*Colonies on gelatin plates* small bluish points, looking like air bubbles. Superficial colonies after seventy-two hours bluish-white, membranous, 1.4 mm. in diameter, deep dark violet color; after fourteen days, almost black. Very slight liquefaction.

*Gelatin Stab.*—Pale violet growth after twenty-four hours, later the membrane is deep, dark violet, the zone of liquefaction takes on a dark violet color, with a dark violet sediment at the bottom of the stab. Fine brownish floccules in the middle of the stab.

*Litmus Sugars* (Hetsch's media).—*Grape-sugar*, pale red color, violet membrane. *Maltose*, the same. *Mannite*, medium not changed.

On all other media, and on potato, deep violet to black violet pigmentation with membrane formation.

Bampton: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1913, lxxi, 129.

### CHROMOBACTERIUM JANTHINUM (Zopf)

Described originally by Zopf[1] as *Bacillus janthinus*. In America isolated by Jordan[2] from sewage and water at Lawrence, Mass., and by Wright[3] from the water of the Schuylkill River. Present description from Wright.

**Morphology.**—A medium-sized, generally short, motile bacillus, with rounded ends, sometimes growing in long forms and threads; it has a polar flagellum; no spore-formation observed.

**Cultural Characters.**—Grows best at room temperature. No growth at 35° to 36° C.

*Colonies in Gelatin.*—On the second or third day the surface colonies are thin, translucent, slightly opalescent expansions, with irregular, sharply defined outlines, and a diameter of several millimeters; toward the center a grayish haziness may be seen. Under a low power the colony is thin and translucent, and shows very numerous fine lines, running in various directions and sometimes branching; the outline is irregular, but sharply defined. Toward the center the colony has a slightly yellowish tint, and there may be a refractive central nucleus of the same color. The deep colonies are rounded or slightly irregular, brownish in color, finely granular, with smooth, sharply defined outlines. The surface colonies soon become grayish white throughout and denser; later a violet color appears, generally at the center, and the colony begins to sink into the gelatin, which is slowly liquefied beneath it. After a few days the colonies have a deep black-violet color, and are sunken in the gelatin, the liquefaction of which never spreads very far beyond the colony.

*Gelatin Slant.*—A deep furrow formed, lined by a membrane, at first grayish and later violet in color.

*Gelatin Stab.*—At first a smooth, shining, grayish-white surface expansion, which spreads rather widely and has irregular margins; this soon becomes violet in color and sinks in the gelatin, forming, after some time, a deep, rather irregular excavation, lined by a violet-colored membrane, beneath which slow liquefaction occurs; the excavation is usually narrower at the top, so that when it has reached the tube wall a ring of non-liquefied gelatin remains above; the liquefied gelatin is somewhat clouded, and there is some grayish or violet-colored sediment; there is a fair growth in line of inoculation of confluent brownish colonies.

*Acid Gelatin.*—Good growth with an alkaline reaction.

*Agar Slant.*—Grayish, semitranslucent, smooth, shining growth, with wavy, sharply defined outlines, spreading rather widely below; this later becomes deep violet in color and membranous in character.

*Bouillon.*—Turbidity; a dark, violet-colored membrane forms on the surface; after a time the bouillon acquires a smoky violet tint and there may be a violet-colored sediment.

*Potato.*—Dense black-violet, membranous, coherent layer, sometimes shining, and in some places having a granular appearance, due to minute and numerous delicate wrinkles.

*Litmus Milk.*—Decolorization with the formation of a dense, coherent, wrinkled, dark violet-colored membrane on the surface. The milk acquires a violet color; there is no coagulation, the reaction is alkaline.

*Sugar Gelatin in Deep Stab.*—Fair growth of discrete and confluent colonies; no gas is produced.

*Rosolic Acid.*—Color is deepened; a violet-colored membrane forms on the surface.

*Indol Production.*—Negative or doubtful.

1. Zopf: Die Spaltpilze. 1885, p. 68.
2. Jordan: Rep. Mass. State Board of Health, Part II, 1890, p. 840.
3. Wright: Mem. Nat. Acad. Sc., 1894, vii, 422.

### CHROMOBACTERIUM CŒRULEUM (Smith)

Found in the water of the Schuylkill River by Smith[1] and by Wright.[2] Present description from Wright.

**Morphology.**—A rather small, motile bacillus, with rounded ends occurring single, in pairs, and occasionally in long forms; it has several flagella, probably normally about four; no spore-formation observed.

**Cultural Characters.**—*Colonies in Gelatin.*—About the third day the surface colonies are thin, translucent expansions, with irregular, sharply defined outlines, and a diameter of about 2 mm.; they have a slate-blue color. Under a low power they are thin and finely granular, and in younger colonies, a few delicate branching lines may be observed. The outlines are irregular and leaf-like, and sharply defined. The deep colonies, under a low power, are irregularly oval, finely granular, slightly yellowish or brownish toward the center, and sharply defined in contour. The surface colonies may increase somewhat in size, grow thicker and more bluish in color, and slowly liquefy the gelatin. About the fifth day they may appear as rounded, bluish-gray masses, with margins no longer sharply defined, lying around, saucer-shaped depressions, containing clear liquefied gelatin, which surrounds the colony on all sides. Under a low power the colony is made up of dense, dark brown masses, and is rather opaque and coarsely granular; the margin is ragged and broken, and in the liquefied gelatin around it detached clumps are seen. The liquefaction proceeds slowly for some distance around the colonies and forms a wide zone, if the colonies are not numerous.

*Gelatin Stab.*—Liquefaction in deep saucer form, constricted at the top and inclosing an air space. On the surface of the liquefied gelatin a bluish-gray membrane; the liquefied gelatin is clouded, and there is a bluish sediment; there is some development in the line of stab; the liquefaction is slow, and practically ceases when about a fourth of the gelatin in the tube has been liquefied.

*Acid Gelatin.*—Grows well.

*Agar Slant.*—A slate bluish, smooth, shining, narrow stripe, with wavy margins; growth is rather dense by transmitted light; after a short time the bluish color gives place to a gray.

*Bouillon.*—Clouded and whitish flocculent sediment; a few scattered bluish flocculi may be seen on the surface, and there may be a bluish ring on the tube wall at the level of the liquid.

*Potato.*—Slate-blue, irregular, dense, somewhat viscid-looking growth; in older cultures becomes dirty brown in color.

*Litmus Milk.*—Coagulated and decolorized; the clot is firm and the cloudy serum is bluish, with blue flakes in it; there is a blue ring on the wall of the tube; the reaction is neutral.

*Sugar Gelatin in Deep Stab.*—Fair growth; no gas production.

*Rosolic Acid.*—Color is deepened.

*Indol Production.*—Marked reaction with the coincident production of nitrites.

*Relation to Temperature.*—Grows well at the temperature of the room and at 35° to 36° C.

1. Smith: Med. News, 1887, ii, 758.
2. Wright: Mem. Nat. Acad. Sc., 1894, vii, 422.

## ENVIRONMENTAL BACTERIA PRODUCING A YELLOW PIGMENT

### Liquefying

*Bacterium luteum* List.
*Bacillus fulvus* Zimmermann.
" *arborescens* Frankland.
" *aquatilis* Frankland.

Non-liquefying

*Bacillus fuscus* Zimmermann.
" *flavus* Fuhrman.
" *subflavus* Zimmermann.
" *aurantiacus* Frankland.

### BACTERIUM LUTEUM (List) Adametz

Found in the stomach contents of sheep by List[1] and in water by Adametz.[2]

**Morphology.**—Elliptical cells 1.1 to 1.3 $\mu$ in length.
**Motility.**—Negative.
**Cultural Characters.**—Grows at room temperature on ordinary media.
*Gelatin Plates.*—Irregular surface colonies slimy in consistency, orange yellow in color. Under low power consisting of many club-shaped coarsely granular zoöglœal masses, each of which is made up of several segments. No liquefaction.
*Gelatin Stab.*—Development slow along line of inoculation with an orange-yellow layer on the surface. No liquefaction.
Carbohydrates not fermented.
*Milk.*—At 30° C. a pigmented layer on the surface in twenty-four to thirty hours with a pale yellow color in the milk below. Later coagulation of the casein and separation of a watery whey.
Orange-yellow pigment, soluble in water, alcohol, and ether. Not changed by alkalies. At once destroyed by acids.

1. List: Untersuchungen über die in und auf dem Korper des gesunden Schafes vorkommenden niederen Pilze. Inaug. Dissert. Leipzig, 1885, p. 53.
2. Adametz: Mitteil. d. österr. Versuchsstation für Brauerei und Mälzerei in Wien. p. 48.

### BACILLUS FULVUS Zimmermann

Found in Chemnitz water by Zimmermann. Description from Flügge.[1]

**Morphology.**—Short bacilli with rounded ends from 0.88 to 1.3 $\mu$ long and 0.77 broad in single elements, pairs, or in short chains.
No spores.
**Motility.**—Negative.
**Cultural Characters.**—Grows slowly at room temperature, best at 30° C.
*Gelatin Plates.*—Deep colonies irregularly spherical, oval, or elliptical; granular, yellowish gray in color. Usually surrounded by pale yellow patches. Superficial colonies reddish yellow, drop-like. At the end of eight days they measure about 1 mm. in diameter.
*Gelatin Stab.*—Scanty development along the line of inoculation, slightly yellowish in color. On the surface an elevated mass irregularly circular, of the color of gamboge. Liquefaction begins after several weeks.
*Agar Slant.*—An abundant, glistening layer, gamboge yellow in color.
*Potato.*—Slow growth along line of inoculation, at first India-yellow, later ochre-yellow.

Flügge: Die Mikroorganismen. 3. Aufl. 1896, ii, 306.

### BACILLUS ARBORESCENS Frankland

Described originally by the Franklands[1] from the water of the Thames. Present description follows Wright.[2] Isolated from the water of the Delaware River.

**Morphology.**—A medium-sized, slender, non-motile bacillus, occurring often in pairs and indistinctly segmented threads; no spore-formation observed.
**Cultural Characters.**—*Colonies in Gelatin.*—About the third day the surface colonies may be several millimeters in diameter, and consist of yellowish collections of flocculi lying in depressions containing liquefied gelatin, each surrounded by a translucent, almost invisible, hazy zone. The deep colonies are rounded, hazy, translucent spots. Under a low power the surface colonies are seen to consist about their centers of irregular, broken, senitranslucent, yellow, granular masses, while toward the periphery they form thin translucent zones, marked here and there with delicate lines, and lying on the surface of the non-liquefied gelatin. These thin, peripheral expansions in turn are continuous with anastomosing networks or plexuses of numerously branching, thin, delicate, translucent processes of varying widths, which

extend for some distance on the surface of the gelatin. The surface colonies are subject to some variation in appearance, dependent on the gelatin used, but the characteristic thing is the anastomosing peripheral zone, and it will usually be seen more or less well-developed; the deep colonies also vary in their appearance. The typical deep colony, under a low power, when very young and almost invisible to the naked eye, may be seen to consist of a translucent, axial trunk, breaking up at both ends into more or less numerous branches, which, in turn, break up into smaller ones. By the third day the colony has a bushy appearance, consisting of a yellow, semitranslucent, gnarled-looking, central axis, which expands at either pole into a rapidly branching and rebranching tree-like structure. The colony thus seems, in typical cases, to be composed of two symmetrical halves, the outlines of the growth being in general rounded or spherical and well defined. A rather slow liquefaction of the gelatin, which has been permeated by the growth, occurs. In some cases the characteristic bushy appearance of the deep colonies may be absent, and they may be seen under a low power to be yellow, refracting, granular masses, sometimes in pairs, and may be provided with numerous short, irregular, hair-like processes, extending into the gelatin.

*Gelatin Stab.*—Liquefaction in cup-shape or deep saucer form, with haziness along the line of inoculation. The liquefaction extends to the tube wall, and slowly downward, the flow becoming level. The liquefied gelatin is clouded, and yellow in color, with a yellow sediment at the bottom. In the case of the variety B of this bacillus, before mentoned, liquefaction proceeds near the surface in a manner similar to the above, but in addition extends along the inoculation line. The gelatin along the line of inoculation has a hazy appearance for some distance on each side. The liquefied gelatin in both varieties has an alkaline reaction.

*Acid Gelatin.*—No growth.

*Agar Slant.*—Translucent, yellow, iridescent layer, with ill-defined transparent margins.

*Bouillon.*—Clouded; yellowish sediment at the bottom, while on the surface a broken yellowish pellicle forms; the bouillon acquires a yellow tint.

*Potato.*—A warm, orange-colored, viscid-looking, thick, shining, widely spreading layer.

*Litmus Milk.*—Decolorized and slowly coagulated; yellow flakes at the surface; the reaction is neutral.

*Sugar Gelatin in Deep Stab.*—No growth.

*Rosolic Acid.*—Decolorized; reaction alkaline.

*Indol Production.*—Negative or doubtful.

*Relation to Temperature.*—Does not grow at 35° to 36° C.

1. Frankland, G., and Frankland, P.: Ztschr. f. Hyg., 1889, vi, 379.
2. Wright: Mem. Nat. Acad. Sc., 1894, vii, 422.

### BACILLUS AQUATILIS Frankland

Described by the Franklands.[1] Present description from Riley.[2]

**Morphology.**—Rather short rods with rounded ends; in young cultures mostly short oval, but some three or four times as long as broad; in older cultures, rods very long, frequently forming thread-like filaments; occurring for the most part singly. They measure 0.4 to 0.5 by 0.7 to 0.9 $\mu$ in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth dull, pale yellowish, translucent, moderate in amount; margin slightly irregular, lacerate.

*Agar Stab.*—Filiform growth along needle-track, becoming villous at top; small amount of growth on surface, spreading irregularly, very thin and transparent.

*Agar Colonies.*—Round, yellowish, with smooth edge, slightly raised. Under low power, margin smooth; surface very finely granular, with darker portion in center; color yellowish.

*Dextrose Litmus Agar Slant.*—Small amount of growth, very thin and transparent; medium acid; margin irregular, lacerate.

*Dextrose Litmus Agar Plates.*—Colonies small, round, transparent; plate acid.

*Gelatin Stab.*—Filiform growth along needle-track; small amount of growth on surface, irregular, yellowish, translucent; liquefaction slow, beginning only after one to three months, and proceeding slowly; liquefaction crateriform.

*Gelatin Colonies.*—Minute, punctiform, yellowish.

*Broth.*—Liquid clouded, then clear; moderate amount of yellowish sediment, very viscid and stringy; no pellicle.

*Peptone Solution.*—Liquid clouded, then clear, with viscid sediment; no pellicle.

*Litmus Milk.*—Unchanged.

*Potato.*—Small amount of growth, at first very thin and transparent, yellowish, later thick, raised, dark brownish-yellow; rather moist.

*Blood-serum.*—Moderate amount of growth, thin, translucent; color dark, dull yellow, moist, glistening; no liquefaction.

*Fermentation Tubes:*

*Dextrose Broth.*—Arm clear; bulb clouded; small amount of viscid sediment; no pellicle; no gas formation; reaction acid.

*Lactose Broth.*—Arm clear; bulb clouded, with viscid sediment; reaction neutral.

*Saccharose Broth.*—Arm clear; bulb clouded, with viscid sediment; reaction neutral.

**Remarks.**—This organism corresponds with the description given by Migula. Not *Bacillus aquatilis* of Migula which is the organism described by Weichselbaum as *Bacillus aquatilis sulcatus* IV.

1. Frankland, G., and Frankland, P.: Ztschr. f. Hyg., 1889, vi, 381.
2. Riley: Observations on the air organisms in Baltimore. 1915.

### BACILLUS FUSCUS Zimmermann

Found in water by Zimmermann[1] and later by Migula.[2]

**Morphology.**—Straight or curved bacilli with rounded ends about 0.63 $\mu$ in diameter and of various lengths. Irregular in shape and slightly bulging. No spores.

**Motility.**—Negative.

**Stains** well with aqueous and carbolfuchsin. Gram-positive.

**Cultural Characters.**—Grows fairly rapidly only under aërobic conditions at room temperature at 30° C.

*Gelatin Plates.*—Deep colonies punctiform, yellowish-brown. Later they project from the surface as irregular knobby buttons; under low power made up of smaller spherical masses. Under low power the deep colonies are spherical, irregular, granular, grayish-yellow to brownish-yellow; central area surrounded by highly refractive marginal zone. No liquefaction.

*Gelatin Stab.*—Button-like mass at the point of puncture. Later the growth extends over the surface forming a thick, wrinkled layer, at first pale yellow, then chrome-yellow in color.

*Agar Growth.*—Like gelatin growth. Color at first light yellow, then ochre-yellow.

*Potato.*—Dark chrome-yellow, friable growth.

*Broth.*—Slight turbidity. Powdery surface growth which is easily broken and sinks in the bottom and forms a thick chrome-yellow sediment.

Pigment chrome-yellow, soluble in alcohol, destroyed by an excess of acetic and hydrochloric acid.

1. Zimmermann: Die Bakterien unserer Trink- und Nutzwässer. 1 Reihe, 1890, p. 70.
2. Migula: Ztschr. f. Hyg., 1890, viii, 357.

### BACILLUS FLAVUS Fuhrmann

Isolated by Fuhrmann from beer.

**Morphology.**—Organisms from gelatin plates stained with carbolfuchsin are almost egg-shaped, short with rounded ends, length 1 to 1.2 $\mu$, width 0.6 to 0.8 $\mu$. They occur singly, in 2's, but not in chains. The protoplasm is homogeneous, especially in young cultures; older rods stain less intensely, are larger and in the middle almost twice the length and breadth, the protoplasm showing more lightly stained areas. Unstained organisms show no plasma differentiation. Organisms from agar cultures at 20° C. twenty-four hours old are homogeneous, stain intensely and are embedded in a viscid mass which stains pale rose in carbolfuchsin. At this temperature the organisms are extremely small. At 32° to 35° C. the organisms are longer and thicker, but smaller than when cultivated on gelatin. Before division the protoplasm is differentiated, more intensely staining material appearing at the poles, more lightly staining in the center. In old cultures club-shaped involution forms appear. No spores.

**Motility.**—Active like that of *Bacillus subtilis*. Motility most marked in cultures on neutral agar at 32° to 35° C. Stained by Zettnow the organisms show peritrichic flagella, each rod having 3 to 4 long winding delicate cilia five to seven times as long as the organism.

**Staining Reactions.**—Stains well with carbolfuchsin 1 to 3, gentian-violet and other anilin dyes. Both young and old forms Gram-negative. In old club-shaped involution forms small Gram-positive particles appear.

**Cultural Characters.**—*Neutral Gelatin Plates.*—Small, sharply limited, bright yellow colonies, about 1 mm. in diameter in forty-eight hours at 20° C. They are slightly raised in the middle, edges almost round. After forty-eight hours the colonies are 2 mm. in size, somewhat darker in color, surface finely granular and indented. After several days the colonies sink somewhat, the gelatin being softened, but not liquefied. The gelatin dries as it is softened, so that no complete liquefaction appears. The deep colonies are smaller, spherical, dirty, yellowish-white, circumscribed. They show in the interior a radial net-like arrangement.

*Neutral Gelatin Streak.*—In twenty-four hours scanty, dirty-yellow growth, surface moist, homogeneous. This growth increases in size after four to five days; there is a slight liquefaction so that the growth sinks slowly into the medium.

*Gelatin Stab.*—After twenty-four hours at 20° C. growth in the upper part of the stab and on the surface. Later surface growth is moderately thick, dried, yellow, and slightly transparent. Little growth in the depths. After ten to twelve days there is a slight liquefaction with the formation of an air bubble.

*Dextrose Gelatin.*—No gas.

*Optimum growth* on gelatin containing 1 per cent. normal sodium hydrate. No growth with 4 per cent. normal alkali or 1 per cent. normal acetic acid.

*Agar Slant.*—Growth at room and body temperature. Optimum temperature 32° to 35° C. At this temperature after twenty-four hours there is a broad expansion along the line of inoculation, light yellow in color, transparent, surface moist, edges delicately indented. Water of condensation turbid with a light yellow sediment. No film. Later the medium is entirely covered with the growth and the golden-yellow color is increased. In old cultures the growth is deep yellow.

*Agar Stab.*—At room and body temperature scanty development in the line of inoculation with a good growth on the surface. Here a characteristic mass, golden-yellow in color, from which the pigment slowly diffuses into the depths.

*Dextrose Agar.*—No gas.

*Potato.*—In twenty-four hours at 35° C. delicate, honey-yellow expansion, moist and viscid. Later growth is darker in color. The organisms from potato are 2 to 3.8 $\mu$ long and no longer motile.

*Coagulated Egg-albumin.*—Glistening mass in twenty-four hours, egg-yolk-yellow in color, the pigment not being absorbed.

*Neutral Dextrose Broth* (1 to 2 per cent.).—Rapid growth, marked turbidity in twenty-four hours, whitish-yellow sediment. After six days the broth clears and a film forms on the surface, delicate yellow in color with a moderate sediment yellow-white in color.

*Neutral Plain Broth.*—Slow growth at room temperature.

*Peptone Solution.*—Scant turbidity in twenty-four hours at 35° C. No sediment or surface film. Gradually fluid becomes turbid and sediment forms.

Optimum growth in broth containing 1 per cent. normal sodium hydrate, no growth with 2 per cent. normal acetic acid and 5 per cent. normal alkali.

No indol.

*Milk.*—Poor growth. After eight to ten days casein is converted into a soft slimy mass. Gradually the milk clears and the casein is completely dissolved.

Acid production with 1 per cent. dextrose in forty-eight hours equals 2.897 c.c. normal alkali.

Growth under anaërobic conditions scanty without pigment formation.

The pigment can be extracted with water, dilute acids and alkalies, not with fat solvents like chloroform, benzol, ether, alcohol, and acetone. The aqueous solution of the pigment is light yellow, the color not being changed by the addition of sulphuric acid, hydrochloric and nitric acid or sodium or potassium hydrate. It is not changed by heating.

In inorganic nutrient solutions *Bacillus flavus* can utilize asparagin ammonium lactate, ammonium tartrate, ammonium chlorid, and potassium nitrate as a source of nitrogen, provided dextrose, saccharose, lactose, and glycerin are also supplied as the source of carbon. Lactose is utilized less than the other carbohydrates.

*Viability* high, agar and gelatin and potato cultures remaining alive after eight to ten months.

Optimum temperature 32° to 35° C.

Fuhrmann: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1907, xix, 117; 221.

### BACILLUS SUBFLAVUS Zimmermann

Found by Zimmermann in the Chemnitz water.

**Morphology.**—Bacilli with rounded ends from 1.5 to 3 $\mu$ long and about 0.77 $\mu$ broad, united in short chains. No spores.

**Motility** slow.

**Stains** well with hot aqueous anilin fuchsin. Gram-negative.

**Cultural Characters.**—Grows best at room temperature, only under aërobic conditions.

*Gelatin Plate.*—Deep colonies small, yellowish-white, spherical masses. They break through to the surface forming hemispherical, yellowish-white, shining drops which later extend over the surface with an irregular outline. Under low power the surface has a pearly lustre. The colonies gradually become dirty-yellow. No liquefaction.

*Gelatin Stab.*—Thin, yellowish-gray, with finely dentate margins at the point of puncture. Finely indented thread-like growth along line of stab.

*Agar Slant.*—Pale yellow expansion over the entire surface. The color gradually becomes darker, finally becoming pale chrome-yellow to yellow-ochre.

*Potato.*—Scanty, dull, clay-yellow growth.

*Broth.*—Turbidity and fine powdery yellow sediment.

Zimmermann: Die Bakterien unserer Trink- und Nutzwässer. 1 Reihe, 1890, p. 62.

### BACILLUS AURANTIACUS Frankland

Found in well water by the Franklands.

**Morphology.**—Short, thick bacilli varying greatly in dimensions, sometimes in pairs, often in long thin filaments. Individuals 1 to 7 $\mu$ long, half as broad.

The organism show, polar staining.

**Motility.**—Slight. In hanging drops the organisms tend to form thick coherent clumps. No spores.

**Cultural Characters.**—Grows slowly at room temperature on the usual media.

*Gelatin Plates.*—Superficial colonies homogeneous, opaque, elevated, pale orange in color. Deep colonies spherical, granular, with smooth edges. No liquefaction. Under low power the superficial colonies are dark and structureless, the deep granular disks with smooth edges. No liquefaction.

*Agar.*—Orange-colored expansion, almost limited to line of inoculation.

*Potato.*—Thick growth along line of inoculation, shining orange-red color.

*Broth.*—No turbidity. Thin surface film with occasional orange particles in it. Pale orange-colored precipitate. Slight reduction of nitrates to nitrites. Considerable ammonia formed from peptone.

Frankland, G., and Frankland, P.: Ztschr. f. Hyg., 1889, vi, 390.

## ENVIRONMENTAL ORGANISMS PRODUCING BROWN, BLACK, OR GRAY PIGMENT

### Liquefying

*Bacillus glaucus* Maschek.
" *ferrugineus* Rullman.

### Non-liquefying

*Bacillus fuscescens* Migula.

### BACILLUS GLAUCUS Maschek

Found in water. Description from Flügge.

**Morphology.**—Slender bacilli of various length.

**Motility** lacking.

**Cultural Characters.**—Grows rapidly at the room temperature on the usual culture media.

*Agar Slant.*—Rapid development of a grayish expansion.

*Gelatin Plates.*—Round, gray colonies with sharply defined outlines, the centers becoming intensely gray on the fourth day, the margins brown and folded radially. By the end of the eighth day liquefaction has set in and the colonies sink to the bottom.

*Gelatin Stab.*—Rapid development along line of inoculation and on the surface. The entire tube is liquefied with a gray deposit at the bottom.

*Potato.*—Growth at first dirty-white, later slimy and dark gray in color.

Flügge: Die Mikroorganismen. 3. Aufl. 1896, ii, 313.

### BACILLUS FERRUGINEUS Rullmann

Isolated by Rullmann from the city water in Munich.

Actively motile bacillus with slightly rounded ends. In sugar broth at 22° C. 0.8 $\mu$ by 2.2 $\mu$ in size, without polar staining. On potato at 22° C. 0.5 $\mu$ by 1.4 $\mu$ with frequent polar staining. In Winogradsky solution at 30° C. the organisms are smaller than on potato, measuring about 0.2 $\mu$ wide and are enclosed singly, in pairs, or more, in a capsule 1 $\mu$ wide and 1.2 $\mu$ long. On nitrite agar short rods are formed 0.3 $\mu$ broad and 0.8 $\mu$ long. Involution forms of large size on meat extract agar without peptone, at 37° C., 1.2 $\mu$ wide and 7 $\mu$ in length.

Growth on ordinary media active and characterized by the development of a brownish pigment. Colonies on *gelatin plates* show brown pigmentation in the first day or two. On *beerwort agar* the colonies are large and round with dark brown centers surrounded by light brown zones. On *blood-serum* the pigment is first light yellow, then seal red, and the medium is liquefied. On *meat extract glycerin agar* a greenish fluoresence diffuses through the entire mass after several days. *Gelatin* liquefied in forty-eight hours with intense brown pigmentation. No fermentation in *sugar agar stabs*. In *milk* the serum is slightly colored, the layer of fat appears dark yellow. The diffusible pigment is soluble with difficulty in water, ordinary alcohol, benzol, and petrolether, easily soluble in acid and alkaline alcohol and acetone.

Non-pathogenic to mice.

Van Iterson, Jr., claims that this species is capable of splitting cellulose.

Rullmann: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1898, xxiv, 465.

### BACILLUS FUSCESCENS Migula

Originally described as *Bacillus fuscus limbatus* from bad eggs by Scheibenzuber.[1] Later the same organism was found by Tataroff in water. Named by Migula.[2]

**Morphology.**—Short, actively motile bacilli, rarely arranged in short chains.

**Cultural Characters.**—*Agar Slant.*—Superficial expansion gradually spreading over the entire surface. The agar beneath takes on a brown color.

*Gelatin Plates.*—Small, brownish, circular colonies frequently surrounded by a lighter zone two or three times as broad. No liquefaction.

*Gelatin Stab.*—Delicate growth on the surface. Thread-like growth in the stab with short branches from which small, round projections develop. The growth looks like a saw with toothed edges. The gelatin near the line of growth takes on an intense brown color. The pigmented zone is sharply separated from the colorless zone and takes on the form of a sack, gradually widening toward the bottom.

*Potato.*—Brownish expansion.

Non-pathogenic.

1. Scheibenzuber: Allg. Wien. med. Ztg., 1889, xxxiv, 171.
2. Migula: p. 836.

## ENVIRONMENTAL BACTERIA PRODUCING WHITE OR COLORLESS COLONIES

Rarely the white color shades off into pale yellow or pale brown.

Liquefying

*Bacillus candicans* Frankland.
" *delicatulus* Jordan.
" *hyalinus* Jordan.

Non-liquefying

*Bacillus albus* Eisenberg.
" *aquatilis* Migula.
" *arborescens non-liquefaciens* Ravanel.

### BACILLUS CANDICANS Frankland

This organism was first found by the Franklands[1] in soil. The species described by Tataroff[2] as "weisser Bacillus" is probably the same. Jordan's[3] *Bacillus ubiquitus* differs only in its power to reduce nitrates. Present description from Riley.[4]

**Morphology.**—Very short rods, with rounded ends, frequently appearing spherical; occurring for the most part singly and in pairs. They measure 0.6 by 0.7 to 1 $\mu$ in twenty-hour cultures on plain agar.

**Motility.**—Non-motile.

**Staining Properties.**—Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth white, thin and translucent at first, later opaque; growth moderate in amount spreading very little beyond needle-track; margin regular; surface smooth, glistening.

*Agar Stab.*—Filiform growth along needle-track; small amount of regular, white growth on surface.

*Agar Colonies.*—Round, white, margin regular. Under low power—margin smooth; color very dark gray.

*Dextrose Litmus Agar Slant.*—Small amount of growth, very thin and transparent; color white; medium acid.

*Dextrose Litmus Agar Plates.*—No growth visible.

*Gelatin Stab.*—Filiform growth along needle-track; small amount of regular, white growth on surface; no liquefaction.

*Gelatin Plate.*—No growth visible.

*Broth.*—Liquid clouded, with small amount of viscid sediment; no pellicle.

*Peptone Solution.*—Liquid faintly clouded, with small amount of viscid sediment; no pellicle.

*Litmus Milk.*—Unchanged.

*Potato.*—No growth. Thick, brown growth described by Migula.[5]

*Blood-serum.*—Moderate amount of growth, moist, glistening; color white, translucent; no liquefaction.

*Fermentation Tubes:*

*Dextrose Broth.*—Arm clear; bulb clouded; small amount of friable sediment; reaction acid; no gas formation; no pellicle.

*Lactose Broth.*—Arm clear; bulb clouded; small amount of friable sediment; reaction neutral.

*Saccharose Broth.*—No growth visible; reaction neutral.

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1. Frankland, G., and Frankland, P.: Ztschr. f. Hyg., 1889, vi, 397.
2. Tataroff: Die Dorpater Wasserbakterien. 1891, p. 37.
3. Jordan: Report State Board Health, Mass. 1890, p. 830.
4. Riley: Observations on the air organisms in Baltimore. 1915.
5. Migula: p. 425.

### BACILLUS DELICATULUS Jordan

Found by Jordan[1] in the effluent from Lawrence sewage.

**Morphology.**—Medium-sized plump bacilli 1 $\mu$ broad and 2 $\mu$ long, in pairs and in short chains. No spores.

**Motility.**—Very active.

**Cultural Characters.**—*Grows* better at 37° C. than at 22° C. No growth below 15° C. Obligate aërobe. The cultures die in several weeks.

*Agar Slant.*—Grayish, wrinkled expansion which later becomes glistening porcelain white.

*Gelatin Plates.*—Colonies whitish, homogeneous, with regular radiating edges. Liquefaction in forty-eight hours, the colonies showing darker centers and lighter peripheries.

*Gelatin Stab.*—Rapid growth along line of inoculation, liquefaction beginning in forty-eight hours. By the end of seven days the gelatin is completely liquefied and cloudy. A thick, whitish surface scum is formed with a heavy, brownish, flocculent precipitate. Growth good in both slightly acid and slightly alkaline gelatin.

*Potato.*—Gray, spreading, flat growth.

*Milk.*—High acidity and firm coagulation.

*Broth.*—Turbidity rapidly produced with scum and white precipitate.

*Nitrates* reduces to nitrites rapidly and vigorously.

Bergey[2] states that this species is Gram-negative, has peritrichous flagella, and does not produce indol.

1. Jordan: Report Mass. State Board Health. Part II, 1890, p. 837.
2. Bergey: p. 137.

### BACILLUS HYALINUS Jordan

Found by Jordan[1] in the sand of a filter tank at Lawrence.

**Morphology.**—Long, large, plump bacilli with rounded ends and usually in short chains, measuring 1.5 $\mu$ broad by 3.6 to 4 $\mu$ long. No spores.

**Motility.**—Active. According to Bergey[2] the flagella are peritrichous. Grows as a facultative anaërobe, better at 37° C. than at 21° to 23° C.

**Cultural Characters.**—*Agar Slant.*—Spreading, dull gray expansion on the surface, thin and rather tough, rapidly growing. Small warty projections appear in four to five days.

*Gelatin Plates.*—Colonies grow rapidly appearing in twenty-four hours. They are hazy with dark centers surrounded by broad translucent zones. Under low power they appear

coarsely fibrillar in the middle, with short fibrils radiating from the edge. At the end of forty-eight hours the colonies are 1 to 1½ cm. in diameter, evenly round, with translucent centers and distinct, opaque, yellowish rims with fibrils radiating from the edges.

*Gelatin Stab.*—Growth rapid along line of inoculation in long, narrow, funnel-shaped liquefaction. The liquid gelatin is cloudy with a precipitate at the bottom. In about eight days a lustrous, tenacious, white surface scum is formed, the gelatin is completely liquefied and perfectly transparent with a white flocculent deposit at the bottom.

*Potato.*—Spreading, dry, grayish-white growth in two to four days with small protuberances appearing later on the surface.

*Broth.*—Turbidity with thick pellicle and stringy precipitate.

*Milk.*—Acidity and firm coagulation.

*Nitrates* reduced vigorously and rapidly. Indol not formed (Bergey).

1. Jordan: Report State Board Health of Mass. 1890, p. 836.
2. Bergey: p. 138.

### BACILLUS ALBUS Eisenberg

First described by Eisenberg[1] in 1891. Common in water. The same organism possibly described by Maschek[2] as "Der weisse Bacillus."

**Morphology.**—Short bacillus with rounded ends often in short chains.

**Motility.**—Active.

*No spores.*

*Grows* slowly, only at low temperatures.

**Cultural Characters.**—*Gelatin Plates.*—Round, pin-head colonies. No liquefaction.

*Gelatin Stab.*—Growth slow with a whitish streak in the depth and a white surface pin-head expansion at the point of puncture.

*Agar.*—Milk-white expansion.

*Potato.*—Dirty yellow-white growth limited to line of inoculation.

1. Eisenberg: Bakteriologische Diagnostik. 1891, p. 171.
2. Maschek: Die Bakterien der Trink- und Nutzwässer, Adametz. Vienna, 1888.
3. Migula: p. 786.

### BACILLUS AQUATILIS Migula

Described by Weichselbaum as *Bacillus aquatiiis sulcatus* IV in the Vienna water with *Bacillus aquatilis sulcatus* I. Named by Migula.

**Morphology.**—Short rods which grow out into long threads in broth.

**Motility.**—Active in short rods. Threads non-motile.

*No spores.*

*Gram-negative.*

**Cultural Characters.**—*Agar.*—Little development at 37° C. At 22° C. a gray-white expansion in forty-eight hours.

*Gelatin Colonies.*—Surface colonies appear in four days with thick, white, opaque centers and thin bluish edges with serrated edges. Under low power striated, the small colonies being white and the larger colonies yellow in the centers. Later the colonies are larger and thicker with irregular furrows. Deep colonies round and yellow. No liquefaction.

*Gelatin Stab.*—Growth slow. Thin gray-white expansion with serrated edges up to the wall of the tube.

*Potato.*—No growth.

### BACILLUS ARBORESCENS NON-LIQUEFACIENS Ravanel

Isolated by Ravanel from made earth at depth of 2 feet.

**Morphology.**—Straight, slender rods, with rounded ends, from seven to thirteen times as long as broad. Occurs singly and in chains of several elements. Spores not demonstrated.

*Characters.*—Strict aërobe. No growth in an atmosphere of hydrogen.

*Motility.*—Rods turn almost end for end and back again. No progression noticed.

**Cultural Characters.**—*Colonies on Gelatin Plates.*—Visible at end of forty-eight hours, as bluish, indistinct, cloudy dots, easily overlooked. The deep colonies cannot be distinguished from those on surface. They resemble the colonies of *Bacillus ramosus*, but are less distinct, smaller and not so white, nor do they grow so rapidly. Each colony consists of an axial trunk, both ends of which split up into numberless fine lines, somewhat interlaced, but on the whole radially disposed. These terminal branches are six to seven times as long as the trunk itself. In some colonies the trunk splits into several large branches, each of which gives rise to many

fine ones, the whole giving a star-shaped figure. Others are made up of several sticks loosely laid together, each one of which splits up into fine branches at both ends; while others again are like the branch of a tree, one end breaking into smaller branches, which they break up into finer lines, while the other end is larger, and breaks up directly into the fine brush-like filaments. After four days the colonies become more plainly visible, like bluish clouds. Under the low power (80 diameters) the fine terminal lines have increased very much, so that the entire mass assumes a round shape. In many there are two foci, as it were, formed by the ends of the trunk where the branching first began. The lines increase for some time, growing longer and becoming more tangled, making the colony more dense in appearance, but even after two weeks they look only like bluish, cottony masses, none more than 1 mm. in diameter, and to the naked eye showing the bipolar arrangement. There is never any liquefaction perceptible. Gelatin colonies closely resemble those of *Bacillus arborescens* of Frankland.

*Agar Slant.*—Growth is barely perceptible until the third day, when it is found to have spread quite widely over the surface. It resembles the growth of the bacillus of influenza very closely. Along the needle stroke is a faint colorless line, and on either side may be seen, by close inspection, colonies which resemble drops of moisture. They are 0.5 mm. in diameter, colorless, and always discrete. The water at bottom shows a whitish growth. After three weeks the growth becomes somewhat more opaque, but never takes on color, and is always hard to see. It is best seen by transmitted light.

*Gelatin Stab.*—On second day a faint whitish growth is seen on surface and along the stab. The surface growth spreads until about the sixth day, when it is 3 mm. in diameter, and is irregular in outline and thickness, piling up at the center. It looks as though layer after layer had grown from the center on the surface of the one preceding, each being of irregular outline, and extending a lesser distance from the common center. It acquires a white metallic lustre about the same time. Along the stab fine outgrowths appear about the fifth day, while deeper colonies appear as separate, minute, white dots, which extend to bottom of puncture.

*Potato.*—No growth can be seen until the seventh day, when it acquires a somewhat different color from the potato, which later (sixteen days) becomes about the color of honey. Until the seventh day, only a moisture can be seen. It is a very thin layer, smooth, moist, and looks glazed.

*Bouillon.*—On third day a slight diffuse cloudiness is seen, which increases somewhat, but never becomes dense. Later the growth settles to the bottom, leaving liquid almost clear.

*Rosolic Acid.*—Very slight growth and no change of color.

*Litmus Milk.*—Color is discharged to some extent by the third day. About the tenth day a reddish tint is seen, and the casein is coagulated in flocculi. Reaction acid. There is no subsequent digestion of the casein up to five weeks.

*Sugar Gelatin, Deep Stab.*—No growth seen for a week, when a single colony developed, deep white and sending out fine branches.

*Gelatin Slant.*—At first a faint whitish line, 1 mm. wide, along needle mark. Later this became an elevated white band, 2 mm. wide, with uneven surface and irregular edges. No liquefaction after three weeks.

Indol negative. Relation to temperature—more rapid at 35° to 36° C.

## Tribe 2. Erwineæ, Nov. Trib.

Plant pathogens. Growth usually whitish, often slimy. Indol generally not produced. Acid usually formed in certain carbohydrate media, but as a rule no gas.

### GENUS 3. ERWINIA Committee

Character those of tribe.

Type species, *E. amylovora* (Burrill, Trevisan) Com.

#### Bacillus amylovorus (Burrill) Trevisan

Originally reported by Burrill[1] as *Micrococcus amylovorus*. The cause of pear blight. Description from Erwin Smith.[2]

**Morphology.**—Rod-shaped organisms often 3 to 6 $\mu$ long. No spores.

**Staining Reactions.**—Stains easily with the usual anilin dyes. Not acid-fast. Gram-negative.

**Motility.**—Active, due to peritrichic flagella stained by Moore's modification of Loeffler's stain or by van Ermengem's stain.

**Cultural Characters.**—Cultivated easily on ordinary media. Optimum temperature 30° C. Growth at 3° C. is very slow. No growth at 0.5 C. Aërobic and facultative anaërobe.

*Agar Plates* (peptone beef agar).—Circular, small, more or less opalescent, white surface colonies with entire margins. Deep colonies smaller, sometimes ringed, fringed or hazy margins. On thinly-seeded plates of agar with a reaction of +15 the surface colonies are homogeneous, wet, and shining. They may reach a diameter of 5 or 6 mm. by the end of the sixth day. Growth on agar may be butyrous (D. H. Jones).

*Agar Stab.*—Good growth along entire length of stab.

*Gelatin Plates.*—Colonies circular and tend to pile up rather than to spread widely. The gelatin is liquefied, a pit of liquefaction often appearing rather promptly about the colony. Liquefaction usually rather slow. Growth best on gelatin when it is made neutral or nearly neutral to phenolphthalein by sodium hydrate.

*Milk.*—No change in color. After some days a soft curd settles and later this is more or less completely digested. If a lab-ferment is produced it must be very sensitive to heat (fifteen minutes at 57° C.).

*Litmus Milk.*—No reddening. Casein precipitated in a few days. There is often a partial reduction of the litmus, the medium being bluer at the top than the control, paler blue at the bottom and wholly reduced in the middle, *i. e.*, changed to a pale brownish-white.

*Bouillon.*—Rapid growth with a prompt and heavy clouding and a slight granular pellicle if the tubes are not disturbed. Rapid growth in sugar broth. In potato broth the same appearances as in beef bouillon.

*Potato juice* in fermentation tubes. Cloudy growth in closed arm without gas.

*Indol* reaction scanty or negative. No odor from cultures. Acids formed from various carbohydrates, glucose, levulose, saccharose and maltose. No gas. Tolerant of small quantities of malic acid and citric acid, but not of lactic acid. No destruction of starch.

No growth in Uschinsky's or Cohn's solution.

Thermal death-point in broth lies between 45° and 50° C. (D. H. Jones). In liquids all organisms killed by an exposure to 55° C. (O. Gara).

Resists drying on coverslips up to five days (L. R. Jones, D. H. Jones) and freezing.

*Pathogenic Action.*—Identified easily by streaking pure cultures on raw green pears which should rot, and on ripe pears which should not rot. The organism is the cause of blight in the pear, apple, quince, plum, loquat, cherry, apricot, strawberry, and in certain wild genera. The pathological process begins by a destruction of the blossoms, green fruit, and young shoots, including young leaves which are especially favorable places for its development. The disease passes quickly downward, chiefly by way of the bark parenchyma, into the inner bark of the larger branches and of the trunk which often are girded and killed. The blighted branches are conspicuously black and brown, the dead persistent leaves looking as if they were scorched. The blackening of the leaves is a host reaction and occurs in the absence of the bacteria, but the bacteria often attack leaves as well as stems, passing from the stem through the petiole into the leaf blade. As the fruit ripens and as the inner (living) bark of the shoots becomes firm, in late summer or early autumn, the blight ceases to spread, and the organism, in a majority of cases, dies out in the blighted (killed) trunk and limbs. In a variable per cent. of cases the bacteria persist in certain patches. forming the "hold over blight" of Waite or the "cankers" of Whitzel. These spots ooze a sweetish exudate containing living and virulent bacteria, especially during the increased sap flow of the spring, and are visited by bees and other pollen-gathering and nectar-sipping insects. Through their agency the bacteria are carried to the blossoms of neighboring trees and a new outbreak started. The organisms brought by the insects grow first in the nectar of the flowers or in bitten or punctured shoots.

1. Burrill: Am. Naturalist, 1882, vii, 319.
2. Smith: An Introduction to Bacterial Diseases of Plants. 1920, p. 367.

## Tribe 3. Zopfeæ Com.

Gram-positive rods, growing freely on artificial media. Not attacking carbohydrates.

### GENUS 4. ZOPFIUS Wenner and Rettger

Long rods occurring in evenly curved chains. Gram-positive. Motile. Proteus-like growth on media. Facultative anaërobes. Carbohydrates and gelatin not attacked, hydrogen sulphid not formed.

Type species *Zopfius Zopfii* (Kurth) Wenner and Rettger.

#### ZOPFIUS ZOPFII (Kurth) Wenner and Rettger

Originally obtained by Kurth[1] from the intestinal tract of hens and named *Bacterium Zopfii*. Found frequently by Migula[2] in water. According to Wenner and Rettger[3] the organism described later by Hauser[4]

from decomposing fluids as *Proteus Zenkeri* is the same species. Present description from a strain of *Proteus Zopfii* from the American Museum and from a strain in the Johns Hopkins Pathological Laboratory which is supposed to have come from the original *Proteus Zenkeri*. The two are identical.

**Morphology.**—Long bacilli, measuring 0.5 to 0.6 $\mu$ in width by 2 to 3 $\mu$. in length in young agar cultures. Many of the organisms are much longer, measuring 7 to 8 $\mu$ in length, and chains or longs threads, measuring 15 to 20 $\mu$ are not uncommon. The width remains uniform in these long forms which may be curved or twisted on themselves in various directions. No enlargement on dextrose agar.

**Motility** rather slow and sinuous. Peritrichous flagella.

**Staining Reactions.**—Stains well with ordinary anilin dyes although somewhat unevenly. Strongly Gram-positive.

*Cultivated* on ordinary media as a facultative anërobe. Growth slow, best at 25° C. Some growth at 22° C., growth poor at 37° C.

**Cultural Characters.**—*Plain Agar Plates.*—Surface colonies appear in forty-eight hours, small and delicate, about $\frac{1}{2}$ mm. in diameter, somewhat hazy or cloudy to the unaided eye.

Fig. 69.—*Zopfius Zopfii*, plain agar, twenty-four hours, stained with gentian-violet.

Under low power they are distinctly feathery or appear a little like a spider-web. The centers are dense and the edges irregular and straggling. Deep colonies almost pin-point, under low power showing the same peculiar cobweb appearance. The colonies increase slowly in size and in three to four days are distinctly feathery to the unaided eye. The typical appearance is only shown after ten to twelve days when the colonies are large, 8 to 10 mm. in diameter, with dense centers and radiating peripheries.

*Plain Agar Slant.*—Moist, delicate non-spreading growth in twenty-four to forty-eight hours. In three to four days the edges of the expansion are radiating and irregular. The growth tends to be adherent to the agar. According to Wenner and Rettger inoculations into the condensation water of agar slants (Cantu's method) do not cause spreading.

*Plain Agar Stab.*—Faint line and little surface growth in two to three days. Later the line growth becomes arborescent, with a delicate surface expansion.

*Dextrose Agar Plates.*—Small, delicate, surface colonies in twenty-four to forty-eight hours, $\frac{1}{2}$ mm. in diameter. Under low power they show the typical cobweb appearance with dense centers and straggling peripheries. Deep colonies punctiform, under low power showing disk-shaped or oval centers and feathery edges. The colonies increase slowly in

size and never become large, measuring only 2 to 3 mm. after ten to twelve days. Agar alkaline.

*Dextrose Agar Slant.*—Faint, delicate expansion in two to three days, with arborescent edges. Growth never abundant. Agar alkaline.

*Gelatin Plates.*—Colonies appear slowly, usually after six to seven days, small and delicate, about ½ mm. in size. Under low power they are like agar colonies with dense centers and straggling or branching feathery edges. They increase slowly in size and may be 6 to 8 mm. in diameter after ten to twelve days, with thick heaped-up centers and thin striated margins. No liquefaction.

*Gelatin Stab.*—Faint line and surface growth in two to three days. Later the line growth is distinctly arborescent, with but little surface expansion. No liquefaction.

*Blood-serum.*—Delicate expansion, light brown in color after eight to ten days.

*Potato.*—Delicate, yellowish expansion, appearing after five to six days. The medium may be darkened (Wenner and Rettger).

*Litmus Milk.*—No manifest change.

*Broth.*—Faint turbidity and sediment, appearing only after several days.

*Peptone.*—Faint turbidity and sediment.

*Fermentation Tubes:*

*Dextrose.*—Faint turbidity and sediment in bowl, arm clear. Reaction alkaline.

No fermentation of dextrose, saccharose, or lactose.

Growth poor in egg meat (Wenner and Rettger).

No $H_2S$. Cultures free from odor. No indol.

Non-pathogenic.

1. Kurth: Ber. d. Deutsch. bot. Gesellsch., 1883, i, 97.
2. Migula: p. 815.
3. Wenner and Rettger: J. Bact., 1919, iv, 331.
4. Hauser: Die Bakterien der Fäulnis. 1885.

## Tribe 4. Bactereæ Com.

Gram-negative rods growing freely on artificial media. Generally forming acid from carbohydrates and often gas composed of $CO_2$ and $H_2$.

### GENUS 5. PROTEUS Hauser

Highly pleomorphic rods, filaments, and curved cells being common as involution forms. Gram-negative. Actively motile. Characteristic ameboid colonies on moist media. Liquefy gelatin rapidly and produce vigorous decomposition of proteins. Ferment glucose and sucrose (but usually not lactose), with formation of acid and gas (the latter a mixture of H and $CO_2$).

Type species, *Proteus vulgaris* Hauser.
" *mirabilis* Hauser.
" $X_2$ and $X_{19}$.

#### PROTEUS VULGARIS Hauser

Isolated first by Hauser[1] from putrefying materials. Since then found widely distributed in the intestinal contents of man and animals and in the environment.

**Morphology.**—Small, thin bacilli measuring about 0.5 to 0.6 μ by 1 to 1½ μ on plain agar twenty-four hours old. Many long forms are also present measuring 2 to 3 μ in length. Threads forms are common, measuring 15 to 20 μ, rarely 40 μ. On dextrose agar in twenty-four hours the organisms are much enlarged, measuring 0.6 to 0.7 μ in breadth by 2 to 4 μ in length with longer forms measuring 4 to 6 μ in length.

**Motility** active from 8 to 16 peritrichic flagella. Organisms shoot rapidly from one part of the field to another. No spores.

**Stains** readily by the usual anilin dyes. Gram-negative.

*Cultivated* easily on ordinary media. Best isolated by Cantu's method,[2] the material being planted in the water of condensation on agar slants. After a few hours the growth spreads up the agar in proximity to the water of condensation and may be transferred to fresh tubes. After a few transfers pure cultures are easily obtained. Grows best at 37° C. but grows well at room temperature. Grows well aërobically and anaërobically.

**Cultural Characters.**—*Plain Agar Plates.*—Surface colonies well developed in twenty-four hours, spreading widely from dense central areas and showing outlying, spreading periph-

Fig. 70.—*Proteus vulgaris*, plain agar, twenty-four hours, stained with gentian-violet.

Fig. 71.—*Proteus vulgaris*, dextrose agar, twenty hours, stained with gentian-violet.

eries. Colonies vary in size from 5 to 6 mm. to 2 to 3 cm. depending on their number. They are whitish in the center, rather pale and translucent toward the periphery and often

showing a bluish shimmer by transmitted light. Under low power they are granular, with dense central areas, spreading, branching peripheries and characteristic creeping edges. Deep colonies pin-point, under low power oval or disk-shape. After forty-eight hours the surface colonies are much larger and in three to four days they may spread over the entire plate.

*Plain Agar Slant.*—Luxuriant, thin, moist growth spreading over the entire agar surface in twenty-four hours. It appears shimmering and bluish by transmitted light. After two or three days it increases in thickness and becomes quite opaque. The water of condensation becomes turbid with a flocculent precipitate.

*Plain Agar Stab.*—Abundant line growth in twenty-four hours with a thin surface growth spreading from the point of puncture to the wall of the tube. In older cultures, two to three days, the growth in the depth is more abundant while the surface growth becomes thick and opaque.

*Dextrose Agar Plates.*—Large, spreading, surface colonies in twenty-four hours with dark opaque centers and thin spreading margins. They may measure 1 to 2 cm. in diameter. Deep colonies punctiform. Under low power the surface colonies show darker centers with thin, irregular, fungus-like projections with creeping or spreading edges. Deep colonies oval or disk-shaped. Agar acid with an occasional bubble or bleb of gas. After two to three days the colonies may spread over the entire plate. Reaction of agar permanently acid as a rule.

*Dextrose Agar Slant.*—Thin, moist, smooth, spreading growth in twenty-four hours covering the entire agar surface. Reaction highly acid. In two to three days the growth becomes thick and opaque. Acidity usually permanent.

*Gelatin Plates.*—Colonies grow rapidly and liquefy the gelatin in their immediate vicinity almost at once. Thickly crowded plates are often entirely liquid in twenty-four hours. When the colonies are isolated they are 3 to 4 mm. in size in twenty-four hours, lying in a cup of liquid gelatin. They are thick and opaque in the center and lighter towards the periphery. Under low power they have peculiar cottony centers and peripheral fungus-like prolongations, being surrounded by zones of liquid gelatin into which these prolongations extend. The gelatin is usually completely liquefied in three to four days. Characteristic liquefying colonies develop in 5, 10, or 15 per cent. gelatin. Frequently with 5 per cent. gelatin swarming colonies are found. Here the gelatin is completely liquefied and the colonies are drawn from one place to another by their flagella. Sometimes under low power the contents of the colonies seem to be in violent agitation.

*Gelatin Stab.*—Abundant line growth in twenty-four hours with a cup-shaped liquefaction at the surface. The growth increases in the depth, the liquefaction spreads to the wall of the tube and in two to three days there is a layer of surface liquefaction often $\frac{1}{2}$ inch in depth. The gelatin may be completely liquefied in ten to twelve days.

Kendall, Cheetham, and Hamilton[3] have found that a soluble proteolytic enzyme is produced by *Proteus vulgaris* in plain gelatin. It may be obtained in an active state free from bacteria. The liquefaction of the gelatin is not accompanied by a liberation of ammonia.

*Blood-serum.*—Thick, heavy, white growth along the line of inoculation in twenty-four hours. The serum beneath the growth is rapidly softened and in eight to ten days may be completely liquefied.

*Potato.*—Moist, yellow-brown growth in twenty-four hours spreading over the medium in two to three days. In old cultures it may be quite brown.

*Litmus Milk.*—Faint acidity in twenty-four hours with an alkaline cream ring and a reduction of the litmus below. In two to three days the acidity gives way to an alkaline reaction. The milk first turns pale blue, then thins and turns to a yellowish fluid. By the end of eight to ten days the milk proteins are completely digested. A small amount of gas may be produced (Wolfe[4]) and there may be a consumption of the lactose. Casein is definitely digested as shown by the loss of protein (Wenner and Rettger[5]).

*Broth.*—Turbidity and sediment in twenty-four hours. No scum. Turbidity and sediment increase in intensity in three to four days. Broth does not clear even in old cultures.

*Peptone.*—Turbidity and sediment in twenty-four hours increasing in intensity in four to five days. No scum. No clearing.

*Dextrose Fermentation Tubes.*—Turbidity in bowl and arm in twenty-four hours, with an evolution of gas and an acid reaction. The gas is not usually abundant, the column in the arm averaging about $\frac{1}{2}$ to $\frac{3}{4}$ inch. Of this gas only a small fraction is absorbed by KOH, the balance being explosive. Gas formula $\frac{H}{CO_2} = \frac{3-4}{1}$. Wenner and Rettger state that *Proteus vulgaris* produces 25 to 30 per cent. of gas in dextrose and 2.5 to 3 per cent. of acid in terms of N/20 phenolphthalein as indicator.

*Saccharose* fermented in the same way as dextrose, about $\frac{1}{2}$ to $\frac{3}{4}$ inch of gas accumulating in two to three days with the same formula.

In *lactose* the growth remains confined to the open bulb with a good line of demarcation between it and the closed arm which remains clear. Reaction alkaline. Lactose not usually fermented. Wolfe has shown that lactose is utilized by *Proteus vulgaris*, about 6.9 grams per liter being consumed.

Wenner and Rettger have found that *Proteus vulgaris* ferments levulose, galactose, mal-

tose, and glycerol in addition to dextrose and saccharose. It does not act on lactose, inulin, dulcitol, sorbitol, salicin, raffinose, arabinose, adonitol, dextrin, and starch. Saccharose is sometimes fermented rapidly, sometimes not at all. Maltose may not be fermented. In the present author's cultures, collected from a number of sources in this country and in France, the action on saccharose and maltose noted by Wenner and Rettger has been constantly observed. It may be pointed out, however, that the same strain behaves differently at different times in respect to saccharose. It may ferment it rapidly on one occasion and then very slowly on another, often after six to eight days. The action on maltose seems to be more fixed, some strains fermenting this sugar actively, while some have no action on it.

With plain broth and utilizable carbohydrate "proteus" produces enough acid to bring the hydrogen-ion concentration up to about 5 on the colorimetric scale.

*Cooked Meat.*—Some volatile acid and some gas produced. Little ammonia or amino-acid. No putrefaction. According to Wolfe the gas formula is $\frac{CO_2}{H_2} = \frac{43.6}{56.4}$.

Lead acetate medium containing 0.5 per cent. nitrate agar, 0.05 to 0.1 per cent. lead acetate and 0.2 per cent. glucose gives a brown color with "proteus" growths.

No hemolysis of blood-corpuscles.

Slight growth in Uschinsky and similar synthetic media. More marked when glucose is substituted for glycerol.

Indol positive in about half the cultures. Nitrates reduced to nitrites. $H_2S$ positive. No mercaptan in anaërobic cultures in egg-meat mixtures.

Methyl red positive. Voges-Proskauer negative.

*Proteus vulgaris* has usually been said to have an energetic proteolytic action. It has been shown by Rettger and Newell[6] and by Wenner and Rettger that this organism does not bring about the decomposition of protein material under anaërobic conditions. With milk, egg, meat mixture, and other protein-containing media there is no reduction in the volume of the protein and no foul-smelling products of putrefaction. No or very little mercaptan is produced. Under aërobic conditions the growth of the organism gives rise to non-putrefactive products of protein decomposition. These observations have been confirmed by Wolfe who has shown that *Proteus vulgaris* is not a putrefactive organism like the anaërobes *Bacillus sporogenes* and *Bacillus histolyticus*. It is a moderate gas former, the largest amount of gas being found in cooked meat. Volatile acid production is small and no putrefactive odor is evolved. No indol produced. Wolfe suggests that a slight degree of hydrolysis can diminish the setting power of gelatin and not be a real proteolysis, according to Rettger's definition of putrefaction as the "production of foul odors characteristic of cadaveric decomposition." A powerful urea-splitting ferment is produced, capable of transforming 45 per cent. of the total nitrogen of urine into ammonia.

**Pathogenic Action.**—*Proteus vulgaris* is mildly virulent to animals. A subcutaneous injection of 2 c.c. saline suspension of twenty-four-hour agar cultures produces inflammatory conditions and abscesses which may last several months, the animals showing weakness, loss of weight, and loss of appetite. Some strains are more virulent, killing white rats in eighteen to twenty-four hours by subcutaneous injection. Killed cultures produce local changes in rabbits at the site of inoculation and filtrates of broth cultures are also toxic. Gouget[7] has shown that the direct injection of living or killed cultures of *Proteus vulgaris* into the bile-ducts of dogs and rabbits produces a catarrhal and suppurative angiocholitis. He believes that "proteus" can produce an amyloid degeneration of the liver, from its toxic products. According to Smith and TenBroeck[8] filtrates of this organism contain toxic substances which kill small animals acutely, often in one-half to two hours, by intraperitoneal and intravenous inoculation.

**Distribution.**—*Proteus vulgaris* is widely distributed in the intestines of animals and in the environment. It is frequently isolated from the dejecta. It is often found in decomposing material of animal origin. Obtained by Wenner and Rettger from meat allowed to decompose and from partly decomposed bodies of rabbits and guinea-pigs. In autopsies on man, "proteus" is frequently found in the blood and in the organs as a result of postmortem invasion. It is occasionally found in almost pure cultures in the dejecta of children with summer diarrhea, but has apparently no significance. It is also frequently present in wounds apparently also as a secondary invader. In cystitis it may be an important factor in the production of ammoniacal urine.

### PROTEUS MIRABILIS Hauser

Described by Hauser along with *Proteus vulgaris* as a slow liquefier. Several strains of the author's cultures correspond to Hauser's description. Otherwise typical of "*vulgaris*" they act very slowly on gelatin, producing a liquefaction in eight to ten days, seldom attack blood-serum and may turn milk alkaline without digestion of the proteins. Wenner and Rettger have proposed that the name "mirabilis" be applied to those strains of

"proteus" which ferment saccharose slowly and fail to attack maltose. Since strains may be occasionally isolated which conform to Hauser's original description of "mirabilis" his definition should probably be retained.

1. Hauser: Ueber Fäulnissbakterien. 1885.
2. Cantu: Ann. de l'Inst. Pasteur, 1911, xxv, 307.
3. Kendall, Cheetham, and Hamilton: J. Infect. Dis., 1922, xxx, 251.
4. Wolfe: J. Path. and Bacteriol., 1918–19, xxii, 289.
5. Wenner and Rettger: J. Bact., 1919, iv, 331.
6. Rettger and Newell: J. Biol. Chem., 1912, xiii, 341.
7. Gouget: Arch. de méd. expér. et d'anat. path., 1897, ix, 708.
8. Smith and TenBroeck: J. Med. Research, 1914–15, xxxi (n. s. xxvi), 523.

### PROTEUS $X_2$ and $X_{19}$

Two strains of *Proteus vulgaris* have been described by Weil and Felix[1] which are agglutinated by the blood of patients with typhus fever. Proteus $X_2$ was obtained from the urine of a patient with the disease.

The organisms were short, delicate, Gram-negative rods looking like proteus, slightly motile and forming long threads. They produced blue colonies on Drigalski-Conradi medium, colorless colonies on Endo medium in twenty-four hours, later turning red, fermented dextrose in eighteen hours, lactose in forty hours, caused a peculiar clotting of milk in forty hours, and turned litmus milk-whey red in twenty-four hours, this reaction being like that of the typhoid bacillus. The colonies on plates were like those of *Proteus vulgaris* and gelatin was liquefied in twenty-four hours. The reactions of Proteus $X_{19}$ are practically the same. They have also been isolated from the urine of patients with typhus fever by Kollert and Bauer.[2]

1. Weil and Felix: Wien. klin. Wchnschr., 1916, xxix, 33; 947. Felix: *Ibid.*, 1816, xxix, 873.
2. Kollert and Bauer: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1925, xciv, 432.

## GENUS 6. BACTERIUM Ehrenberg, emended Jensen

Gram-negative, evenly staining rods. Often motile with peritrichic flagella. Easily cultivable, forming grape-vine leaf or convex whitish surface colonies. Liquefy gelatin rarely. All forms except *alcaligenes* and the *B. abortus* group attack the hexoses, and most species ferment a large series of carbohydrates. Acid formed by all (except *B. alcaligenes* and *B. abortus* and others), gas ($CO_2$ and $H_2$) only by one series. Typically intestinal parasites of man and the higher animals, although several species may occur on plants and one (*B. aërogenes*) is widely distributed in nature. Many species pathogenic.

Type species *Bacterium coli* (Escherich).

### BACTERIUM GROUP I

Non-liquefying, fermenting dextrose, saccharose, and lactose with acid and gas.

*Bacterium coli* (Escherich).

### BACTERIUM COLI (Escherich)

Described first accurately and completely by Escherich[1] as *Bacterium coli commune* from the intestinal contents of children, and since then found normally in the intestines of man and practically all animals, as well as in various locations in the environment, as in soil, water, sewage, in food, in milk, etc. The organism noted previously by Emmerich[2] in the stools of cholera patients and known as *Bacillus neapolitanus* was later shown to be identical with Escherich's species, as well as the organisms found by Buchner.[3] *Bacillus coli* has been described under a great variety of names, especially as the cause of a number of diseases in animals in which in reality

Fig. 72.—*Bacillus coli* from plain agar twenty hours old, stained with gentian-violet.

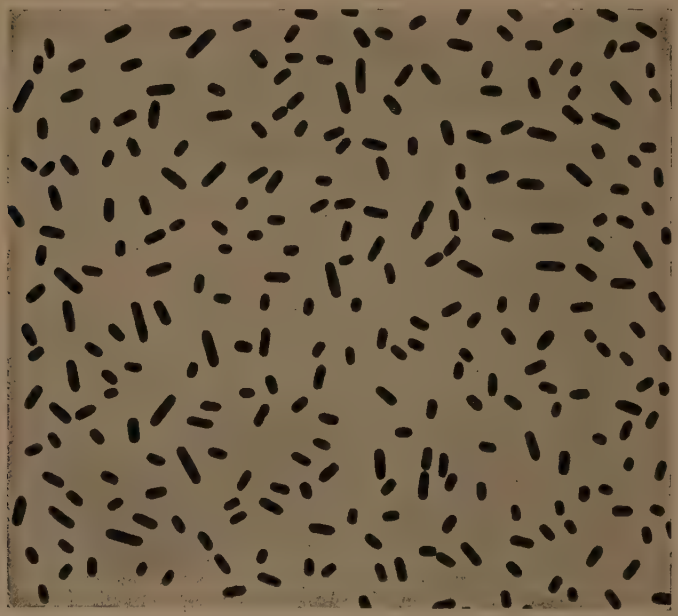

Fig. 73.—*Bacillus coli* from dextrose agar twenty hours old, stained with gentian-violet.

it is a secondary invader and not the etiological agent of the disease in question. It is probably the most widely distributed organism known.

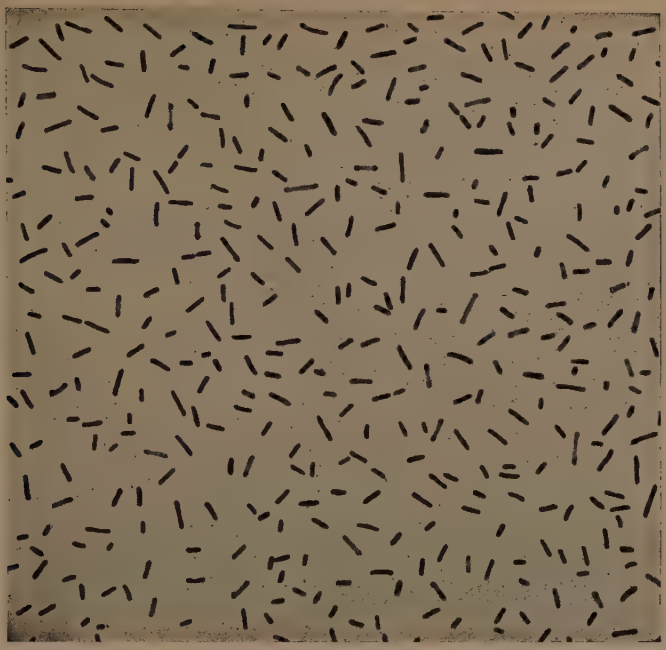

Fig. 74.—*Bacillus coli* showing long forms from plain agar twenty-four hours old, stained with gentian-violet.

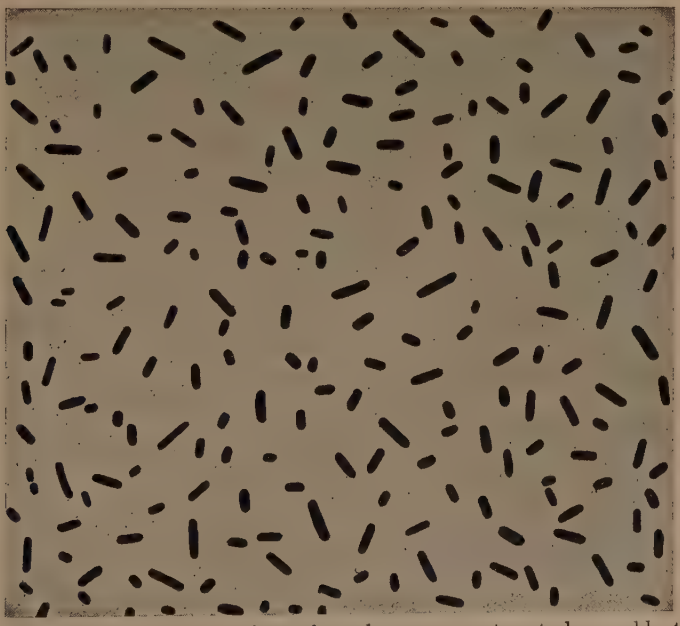

Fig. 75.—*Bacillus coli* showing long forms from dextrose agar twenty hours old, stained with gentian-violet.

**Morphology.**—Small bacilli, measuring on plain agar in twenty-four hours 0.4 to 0.6 $\mu$ in width by 1 to 2 $\mu$ in length. Short, oval, or almost round forms are also common, occasionally appearing almost like diplococci (Adami). Different strains differ greatly in morphology.

Some appear as characteristic, short, stumpy bacilli, some as longer and more uniform rods. Very rarely cultures are encountered which show elongated organisms measuring 0.4 by 2.5 to 3.5 $\mu$ and looking almost like the typhoid bacillus. In many cultures threads measuring 25 to 30 $\mu$ are common and at times the organism grows in shorter or longer chains. The unstained threads show highly refractile polar bodies (Conradi and Bierast[4]). On glucose agar the organisms are definitely enlarged in twenty-four hours, measuring 0.7 to 0.8 $\mu$ in breadth by 2 to 3 $\mu$ in length. This is probably due to the acid.

*Capsules.*—Certain strains of *Bacillus coli* are definitely encapsulated and free from motility. The individual organisms are surrounded by a delicate capsule which may be demonstrated best by Huntoon's stain. When cultivated on the usual media these strains produce a viscid glassy growth a little like that produced by *Bacterium lactis aërogenes*. The cultural reactions, however, indicate that the organisms are true *Bacillus coli*. They ferment the carbohydrates with an excess of hydrogen over carbon dioxid and produce a permanent acidity. They are methyl red positive and Voges-Proskauer negative.

The existence of such encapsulated strains of *Bacillus coli* was first recognized by Smith.[5] In unpublished investigations by the present author in 60 strains of *Bacillus coli* isolated from calves, 10 proved to be encapsulated and 5 similar strains were obtained from other sources.

**Motility** usually slow and leisurely, at times difficult to demonstrate. In many instances motility is entirely lacking. Thus, de Stöchlin found 184 non-motile and 116 motile strains in 300 isolations from human feces. Rarely motility is rapid, not to be differentiated from that of *Bacillus typhosus*. This is especially true in cultures freshly isolated from the intestinal contents.

**Staining Reactions.**—Stains easily by the usual anilin dyes, gentian-violet, methylene-blue, safranin, and dilute carbolfuchsin. Uniformly Gram-negative, decolorizing easily in alcohol.

Flagella may be demonstrated by various methods, van Ermengem's, Loeffler's, Pitfield's, etc. They usually number 4 to 8, have a peritrichic arrangement, and are long and wavy. They frequently but not always appear somewhat shorter than the flagella on *Bacillus typhosus*. With Burri's india ink the organism may show tufts of flagella like those occasionally found on the typhoid bacillus (Gins[7]). It is not yet certain whether flagella actually fail on non-motile strains, but Peppler[8] found them lacking in 19 out of 25 isolations.

**Cultivation.**—*Bacillus coli* may be cultivated on the ordinary laboratory media and is easily accustomed to artificial conditions. The organisms grow best at 37° C. and at temperatures near this, but they grow well, although more slowly, at 18° to 20° C. Little or no growth at 6° to 8° C. or above 45° to 50° C. Barber[9] has found that growth begins at 10° C. and stops at 49° C. Growth is rapid, the length of a generation at 37° C. being seventeen minutes (Barber), twenty-one to twenty-three minutes at 37° C., and seventy-seven minutes at 22° C. (Heheworth[10]). Growth more rapid under aërobic conditions, but anërobic growth usually good. According to Serafini, *Bacillus coli* grows anërobically best in an atmosphere of $CO_2$, next in an atmosphere of $H_2S$. Growth on neutral, slightly acid, or slightly alkaline media.

*Plain Agar Plates.*—Colonies develop rapidly, appearing on the surface and in the depths in twenty-four hours at 37° C. Surface colonies are usually round, slightly elevated, opaque, and measure 1 to 2 mm. in forty-eight hours. Under low power they are granular with dense centers, often nucleated, margins thin, edges entire. Deep colonies are small and punctiform in twenty-four to forty-eight hours, under low power distinctly oval, disk, or whetstone-shaped. The surface colonies may remain small and delicate if the plates are thickly seeded, sometimes being only 3 to 4 mm. after seven to eight days. When the colonies are isolated they grow to considerable size, 8 to 10 mm., are more elevated and thick, and often show very irregular jagged edges. Deep colonies may also increase in size, appearing as distinct ovals or disks, $\frac{1}{4}$ to $\frac{1}{2}$ mm. in diameter.

*Plain Agar Slant.*—Moist, widely spread, flat, opaque growth in twenty-four hours, often with somewhat irregular margins. The water of condensation is turbid with occasionally a delicate film on the surface. In two to three days the growth is much thicker, the turbidity in the water of condensation more marked. The growth increases in intensity, and after eight to ten days the entire agar surface may be covered with a thick opaque mass. In old cultures the growth may become pale brown, especially along the edges. With some cultures of *Bacillus coli* the growth is much more delicate from the start and has a superficial resemblance to a culture of *Bacillus typhosus*.

*Plain Agar Stab.*—Faint line and surface growth in twenty-four hours. The surface growth increases rapidly, spreading from the point of puncture, usually with a crenated edge. It may reach the wall of the tube in six to eight days. The line growth becomes thicker and more opaque.

*Dextrose Litmus Agar Plates.*—Rapid growth, superficial colonies 1 to 2 mm. in size in twenty-four hours, usually round, regular, and uniform. Under low power dense, opaque, margins thin, edges entire. Deep colonies round or oval, $\frac{1}{2}$ mm. in size, under low power ovals and disks. The agar is highly acid in twenty-four hours and may show bubbles of gas, especially about the deep colonies. In three to four days the superficial colonies are larger, 3 to 4 mm. in size, often distinctly nucleated. Deep colonies are also larger, usually distinctly oval, $\frac{1}{4}$ to $\frac{1}{2}$ mm. in size. In plates ten to twelve days old the surface colonies may be 5 to 6 mm. in diam-

eter, flat and smooth, often with crenated edges. The plates become highly acid and do not revert to alkaline.

*Dextrose Litmus Agar Slant.*—Moderate smooth moist growth in twenty-four hours, with turbidity in the water of condensation. Agar highly acid. In forty-eight hours the growth is increased, usually with crenated edges. It often spreads later over the entire surface of the agar slant. The reaction of the agar remains acid even in old cultures.

*Dextrose Litmus Agar Stab.*—Abundant growth in the depths and on the surface. The agar turns red from acid production and is fragmented by bubbles of gas. No reversion to an alkaline reaction.

*Gelatin Plates.*—Colonies of various types develop on the surface in two to three days. Some are small, fine, opaque, about $\frac{1}{4}$ mm. in diameter; under low power granular, round or oval, at times irregular, with distinct edges. Other colonies are more opaque, slightly larger, and distinctly moruloid under low power. In many instances the surface colonies, however, are thin and spreading, somewhat bluish in color, 0.5 to 1 mm. in diameter in two to three days. Under low power these colonies are granular, often show fine radiating veins and irregular fringed edges, sometimes made up of fine spur-like processes. Deep colonies are almost pin-point in forty-eight hours, under low power whetstone or wedge-shaped, yellowish-brown in color. Gelatin colonies never become very large, rarely being more than 2 to 3 mm. even after eight to ten days' growth. With gelatin of low concentration the surface colonies are apt to be thin and spreading in type and may rarely have a superficial resemblance to the colonies of *Bacillus typhosus*. Gelatin not liquefied.

*Gelatin Streak.*—Growth may be thick and opaque, not unlike the growth on slant agar. Rarely the growth is thin and fluid, and has a superficial likeness to the growth of typhoid or paratyphoid bacilli.

*Gelatin Stab.*—Faint line and surface growth in twenty-four hours somewhat increased in forty-eight hours. The growth spreads slowly on the surface and increases in intensity along the line of inoculation. No liquefaction.

Old gelatin cultures show crystals of ammonia, magnesium, phosphate.

*Broth.*—Turbidity and sediment in twenty-four to forty-eight hours with sometimes a fragile scum. In five to six days the turbidity may become dense and cloudy, the sediment much increased. Broth does not clear and the scum does not become marked. Old cultures have a fecal odor and may give a reaction for indol. Occasionally the sediment is viscid.

*Peptone.*—Turbidity and sediment with rarely a fragile scum. Indol positive.

*Blood-serum.*—Fairly abundant smooth growth in twenty-four hours, somewhat increased in forty-eight hours, with crenated edges. In eight to ten days the growth is moderate and flat with crenated edges, often slightly brownish.

*Potato.*—Growth varies greatly, depending somewhat on the character of the potato. In freshly prepared moist alkaline potato it is thick and abundant in twenty-four to forty-eight hours, usually slightly yellow in color. In three to four days it is much increased in amount, of a mealy consistence, and often distinctly brown. In old cultures the brown color is more marked. In many instances the potato growth remains scanty and unpigmented, especially on acid potato.

*Litmus Milk.*—Highly acid in twenty-four hours with a decolorization of the litmus. The acidity increases rapidly, and usually the milk is firmly clotted in forty-eight hours. The coagulation may be later in appearing, and frequently the milk coagulates only on heating. The coagulum is firm and may be red above and white below where the litmus is reduced. The coagulation is usually attributed to the acid reaction, but Konrich[11] and Savage[12] believe that a lab-ferment is also present.

*Lactose Litmus Agar Plates.*—Large reddish colonies with acid production and bubbles of gas. Similar colonies on v. Dregalski-Conradi agar, 2 to 6 mm. in size in twenty-four hours.

*Lactose Litmus Agar Stab.*—Abundant growth along the line of inoculation and on the surface with acid and gas production in the agar.

*Endo Agar.*—Large reddish colonies with gas production.

*Russell's Double Sugar.*—Turbidity and gas production throughout the medium.

*Petrurchky's Litmus Milk-whey.*—Turbidity and production of high acidity, 4 to 5 per cent. of N/10.

Methyl red reaction always positive in typical strains.

Voges-Proskauer reaction always negative in typical strains.

*Smith Fermentation Tubes of Dextrose Broth.*—Rapid growth, turbidity, and heavy sediment in twenty-four to forty-eight hours, with about 1 inch of gas in the closed arm. Gas evolution usually ceases in about three days. The broth is highly acid in reaction. Upon the addition of concentrated potassium or sodium hydrate some of the gas is absorbed, varying from $\frac{1}{5}$ to $\frac{1}{3}$ of the total amount. The balance is explosive. Gas formula $\frac{H}{CO_2} = \frac{3-4}{1}$. In lactose the reaction is practically the same, the gas also consisting of carbon dioxid and hydrogen.

In addition to dextrose *Bacillus coli* ferments other hexoses, like galactose, levulose, mannose, arabinose, and sorbose, the bioses, saccharose, lactose, maltose, melibiose, and treha-

lose, the pentoses, xylose, and rhamnose. Saccharose is first hydrolized to glucose and levulose, which are then fermented, lactose and melibiose to glucose and galactose, maltose and trehalose to glucose and glucose. The trisaccharid raffinose is first hydrolized to melibiose and levulose, the melibiose to glucose and galactose. Various alcohols are also fermented—the 6 atom series, mannite, dulcite and sorbite, the 5 atom series, erythrite and adonite, the 3 atom glycerin.

**Fermentation of Glucose.**—The splitting of glucose is probably due to a bacterial enzyme, which has not yet been isolated. Kuhtz[13] claims that the action is due to the living bacterial cells. The presence of nitrogen is necessary for the reaction. Thus in a solution containing 0.2 per cent. $K_2HPO_4$, 0.5 per cent. NaCl, and 1 per cent. glucose fermentation does not occur. If a little nitrogen (1 mg.) be added fermentation develops. It takes place under both aërobic and anaërobic conditions, better in the air than in an atmosphere of hydrogen (Ducháček), best at 37° C. Various substances are evolved, chiefly carbon dioxid, hydrogen, lactic, acetic, and formic acids.

Other substances produced in small quantities in the fermentation are alcohol, butyric acid, proprionic acid, succinic acid, acetone, and possibly methane.

Harden[14] believes that the anaërobic fermentation of glucose takes place according to the following reaction:

$$\underset{\text{Glucose.}}{2C_6H_{12}O_6} + \underset{\text{Water.}}{H_2O} = \underset{\text{Lactic acid.}}{2\,C_3H_6O_3} + \underset{\text{Acetic acid.}}{C_2H_4O_2} + \underset{\text{Alcohol.}}{C_2H_6O} + \underset{\text{Carbon dioxid.}}{2CO_2} + \underset{\text{Hydrogen.}}{2H_2}.$$

Harden found that under anaërobic conditions the lactic acid corresponded to half the sugar originally present, the alcohol and acetic acid to one-sixth the carbon, and that only small quantities of succinic and formic acid developed.

Both dextrorotatory and levorotatory lactic acid are produced, as well as optically inactive lactic acid. When ammonium salts are present *Bacillus coli* produces levorotatory lactic acid, from peptone media dextrorotatory. Péré[15] reports that *Bacillus coli* from nurslings produces dextrorotatory lactic acid, that from children and adults the levorotatory variety.

The fermentation of dextrose lasts five to six days in 0.5 per cent. glucose where $\frac{3}{4}$ of the sugar is used up. With 1 per cent. solution not half is used up at this time. The optimum concentration of sugar for the reaction is about 6 per cent. With higher concentration, 20 per cent., fermentation does not occur. The acids evolved stop the reaction. When they are neutralized by calcium carbonate ($CaCO_3$) the amount of sugar utilized is increased.

The optimum temperature for the reaction is 37° C., but it occurs at both lower and higher temperatures. Eijkman claims that *Bacillus coli* of human origin ferments 1 per cent. glucose at 46° C. Konrich has shown however that 26 per cent. of 2079 strains did not ferment at 46° C. It has been suggested that strains of *Bacillus coli* from warm-blooded animals ferment at 46° C. while those from cold-blooded do not. This has not been confirmed by Fromme who found 6 strains of *Bacillus coli* from fishes which ferment at 46° C.

In recent painstaking investigations of the members of the "*coli*" group by Clark and Lubs[16] it has been proposed to call the organisms like *Bacillus coli* which produce an excess of hydrogen over carbon dioxid the "low ratio" organisms, those producing an excess of carbon dioxid over hydrogen, like *Bacillus lactis aërogenes* and *Bacillus cloacæ* being known as "high ratio" organisms. In general the low ratio strains are methyl red positive and Voges-Proskauer negative. The high ratio strains have a considerable ability to overcome acid and are in consequence methyl red negative. They also produce acetylmethylcarbinol and give the Voges-Proskauer reaction.

**Fermentation of Lactose.**—In lactose the products of fermentation are much the same as in glucose, consisting of hydrogen, carbon dioxid, acetic, lactic, and formic acids (Buchner). Fermentation is most active in 4 per cent. lactose (Mendel), but higher concentrations can be changed. If calcium carbonate be added to neutralize the acids produced in 5.565 per cent. lactose, all the lactose is fermented (Schmidt).

In addition to the above substances *Bacillus coli* occasionally ferments dextrin, inulin and starch and various glucosides including esculin (van der Leck[17] and Harrison). It decomposes organic acids with the production of hydrogen, carbon dioxid, and methane. The principal organic acids thus affected are formic, succinic, citric, tartaric and their potassium, sodium, ammonium, and calcium salts (van Ermengem and van Lear).

**Decomposition of Proteins.**—*Bacillus coli* decomposes proteins with the production of a number of substances, the most important of which is *indol*. This is formed especially in peptone solutions in the absence of sugar and in the presence of air. It is usually found in twenty-four-hour broth cultures (Kitasato), but may be present in eight hours at 37° C. (Konrich). In certain instances it is found only after fourteen days (Jaffé). The maximum indol production occurs after ten to twelve days (Germano and Maurea). The best solution to demonstrate its production is one containing 10 per cent. peptone, 0.5 per cent. sodium phosphate and 0.1 per cent. magnesium sulphate (Selter) or Dunham's solution containing 1 per cent. peptone and 0.5 per cent. NaCl. Ordinary broth containing 1 per cent. peptone is not adapted to indol formation because of the muscle sugar present (Smith). Trustworthy results can usually be obtained with 5 per cent. peptone.

The presence of indol can be demonstrated by the Salkowski test in which 1 c.c. of 0.02 per cent. potassium nitrite solution and a few drops of concentrated $H_2SO_4$ are added to 10 c.c. of a twenty-four-hour broth culture. Indol gives a rose or a deep red color. Lösener and Wherry regard this quantity of nitrite as frequently too high and say that better reactions are given by adding 1 c.c. of a 0.005 per cent. potassium nitrite solution and 1 c.c. 25 per cent. $H_2SO_4$. After one to two hours at room temperature 2 to 3 c.c. of amyl alcohol may be added. On shaking the indol dissolves in the amyl alcohol which becomes red or rose-colored. Indol can also be shown by Ehrlich's test in which 5 c.c. of solution A (Paradimethylamidobenzaldehyde 4 parts, alcohol 96 per cent. 380 parts, and concentrated HCl 80 parts) and 5 c.c. of solution B (potassium sulphate in saturated aqueous solution) are added to 10 c.c. of the culture. When indol is present a rose-red color develops which is taken up by amyl alcohol.

Nitrates are reduced to nitrites by *Bacillus coli* but in making the test for indol it is always better to add them.

While indol is usually produced by typical strains of *Bacillus coli* certain cultures fail to give the reaction. Thus Konrich has reported 1042 positive indol formers in 2079 strains (50.6 per cent.) and 1037 negative (49.4 per cent.). In general, strains recently isolated from the human and animal body tend to be indol-formers while strains from the environment (soil and water) tend to lose their capacity to produce this substance.

The presence of sugar in the media usually interferes with indol formation. The organisms attack the sugar energetically and the proteins are spared sufficiently to prevent the reaction.

In addition to indol, *Bacillus coli* produces phenol in traces from proteins, tryptophan, and kreatinin. Ammonia is formed from peptone, but not from native proteins (Rettger). Other products of peptone decomposition are hydrogen sulphid, mercaptan, succinic acid, valerianic acid, caproic acid (Blumenthal, Kendall).

**Action on Dyes.**—Bacillus coli has the capacity to reduce dyes, to be traced to reductases. The dyes thus acted on are litmus, rosolic acid, indigoblue, thionin, methylene-blue, toluidin blue, methyl violet, safranin, vesuvin, orcein, neutral red, malachite green and brilliant green. On aëration the color returns. The colorless products (leucobases) arise from the dyes by the entrance of atoms of hydrogen which are removed by the access of oxygen. The most important of these dyes is neutral red which is reduced with the production of a greenish-yellow fluorescence. The reaction takes place at 37° C. in one to five days, more rapidly sometimes at 46° C.

**Hemolysins** or **hemotoxins** were first demonstrated by H. Kayser[18] in fluid cultures of *Bacillus coli*. They appear after two to four days' incubation and are most active against the erythrocytes of dogs, next on horses, beef and rabbit's blood. They have little or no action on the erythrocytes of man, guinea-pigs, sheep, swine, pigeons, and geese. The hemotoxic substance is heat-resistant. A specific antilysin or antihemotoxin can be obtained by immunizing animals. Pribram[19] finds that the hemolysin of *Bacillus coli* can be demonstrated in agar to which ½ to 1 c.c. rabbit's blood is added for each 10 c.c. The solution of corpuscles can also be made out on blood agar plates. With human blood (2 c.c. to 5 c.c. agar) a blue violet or greenish-yellow discoloration of the corpuscles takes place immediately under the colonies (Mandelbaum). Schmidt[20] has shown that hemolytic strains of *Bacillus coli* occurred in 50 out of 73 isolations from typhoid patients and in 11 of 17 isolations from normal individuals. Hemolytic strains of *Bacillus coli* do not differ from non-hemolytic strains in virulence or cultural reactions.

**Destruction.**—Destroyed easily by heat, at 55° C. in one-half to one hour, at 58° C. in about fifteen to twenty minutes, at 60 to 62° C. in ten to fifteen minutes, and at 65° C. in one to five minutes. Fairly resistant to drying. Dried on threads the organisms survive one hundred and fifty-two days in the dark, two hundred and thirteen days in a desiccator over $H_2SO_4$ and one hundred and eighty-three days in a vacuum (Billings and Peckham). The growth is inhibited in agar cultures by a number of metals, copper, brass, iron, silver, zinc, cadmium, and mercury (Bolton); not, however, by platinum, gold, bismuth, nickel, and silicon. Destroyed easily by the ordinary disinfectants, by 1 per cent. carbolic acid, and 1 : 1000 bichlorid of mercury in one to five minutes, etc.

**Pathogenic Action.**—Freshly isolated strains of *Bacillus coli* are virulent to guinea-pigs and rabbits, somewhat less so to mice. In guinea-pigs of average weight, 300 to 400 grams, intraperitoneal or intravenous inoculation of 2 c.c. of a twenty-four- to forty-eight-hour broth culture usually kills the animal in three days. With smaller doses there may be no effect. Large doses sometimes kill in eight to ten hours (Emmerich). With subcutaneous administration larger doses are necessary to bring on a regularly fatal outcome. According to Escherich, 1 c.c. of a twenty-four- to forty-eight-hour broth culture kills guinea-pigs only exceptionally by subcutaneous administration—2 c.c. in about one-third of the animals. Depending on the strain, the fatal dose for guinea-pigs varies from 0.5 to 5 c.c.

Guinea-pigs usually develop a general infection, frequently with gastro-enteritis and with symptoms pointing to lesions of the nervous system. At autopsy the intestines are injected, the lymph follicles swollen, the spleen enlarged, and the peritoneal cavity full of serous fluid. The liver, kidney, heart, and lungs are usually free from lesions. With subcutaneus inocula-

tion, especially with large doses of cultures of low virulence, local abscesses may form. These usually perforate spontaneously. With intraperitoneal inoculation abscesses may form in the peritoneal cavity. In chronic cases the animals develop a peculiar marasmus, slowly emaciating and dying in about six to eight weeks. In early cases of *Bacillus coli* infection the organisms may be isolated in large numbers from the different organs. In late cases the number of the organisms is much less. Occasionally direct injection into the air passages is fatal in one to four days. Coppola[21] reports that guinea-pigs can be infected through the mouth in about 20 per cent. of the cases.

Rabbits are sensitive to *Bacillus coli* in about the same way that guinea-pigs are, dying of septicemia from subcutaneous, intraperitoneal, and intravenous inoculations of large doses of virulent organisms. With subcutaneous injection small doses lead to local abscess formation. When the organisms are introduced intraperitoneally the animals often develop a peritonitis which may be fibrinous, purulent, or hemorrhagic in character. Areas of pus may be found in the peritoneal cavity, a serous-cellular exudate in the pleura or in the pericardium, abscesses in the liver and spleen. As with the guinea-pigs the longer the period between infection and death, the less the number of organisms in the blood and organs.

Direct injection of the organisms into the pleura, pericardium, bladder, and bile-ducts leads to a purulent, fibrinous or hemorrhagic inflammation, pleurisy, pericarditis, pyelonephritis, cholangitis, etc. Blachstein[22] states that rabbits injected intravenously and dying after eight to ten days show small disseminated necrotic foci in the liver with minute concretions in the bile.

A degenerative atrophy of the anterior horns of the ganglion cells has been found by Gilbert and Lyon.[23]

Rabbits cannot be infected by mouth.

White mice are usually immune (Sternberg), but large doses given subcutaneously may lead to general infection or to abscess formation (Neisser). No infection per os. Gray mice are occasionally sensitive to small doses, 0.3 c.c. of a twenty-four-hour broth culture.

In dogs abscesses are common after subcutaneous inoculation (Escherich, Sternberg[24]). These abscesses may rupture spontaneously or be slowly absorbed.

Calves may be infected through the mouth or from the anus (Jensen[25]). Young cats cannot be infected by these methods.

Apes are sensitive to the intracellular toxins of the organism (Emmerich).

In general the lesions produced by *Bacillus coli* are much the same as those which follow injection of *Bacillus typhosus*.

**Virulence.**—Strains of *Bacillus coli* from different sources vary greatly in virulence and a large number are almost totally non-virulent. Escherich and Pfaundler[26] regard fresh strains from diseased intestines as much more virulent than those from normal individuals. Lesage and Macaigne[27] found that the isolations from abnormal intestines were fatal to mice, guinea-pigs, and rabbits in small doses. Among earlier observations Hueppe found that *Bacillus coli* from cholera morbus was very virulent to guinea-pigs by intraperitoneal inoculation, Gilbert and Girode[28] reporting a similar finding. Mellin[29] also found that a strain from a child with intestinal catarrh was very virulent. There are, however, great differences in the virulence of strains of *Bacillus coli* from the same individual and the attempts to correlate virulence with abnormal conditions of the intestines, such as are seen in typhoid fever, are not entirely satisfactory. Strains from definite lesions, however, are apt to be more virulent than those from normal stools (Uhlenhuth[30]). *Bacillus coli* strains of environmental origin, as from water, dust, air, and foods, are almost completely lacking in virulence. Virulence is lost somewhat rapidly on artificial cultivation and may be increased by passage through animals. According to Widal and Besancon and to Nobécourt[31] growth in symbiosis with streptococci and staphylococci causes an enhancement of virulence.

**Toxins.**—*Bacillus coli* does not produce true extracellular toxins. Poisonous substances can occasionally be found in fluid cultures, but these apparently come from the breaking down of the bacterial protoplasm. They are not specific and resemble those produced by other organisms. Killed cultures of *Bacillus coli* are often intensely poisonous to small animals, rabbits, and guinea-pigs.

**Pathogenic Action in Man.**—*Bacillus coli* is present normally in the intestinal tract of man, making its way into the stomach and intestines of very young children and multiplying there indefinitely. Immediately after death the orgnism begins to invade the body and in a short time is almost universally distributed, frequently in association with other intestinal bacteria. In some instances this invasion of the tissues and distribution may begin immediately before death (Zinsser[32]). At autopsy *Bacillus coli* is frequently isolated from the blood and organs irrespective of the disease from which the individual has died. In consequence its mere isolation from the body after death is no proof of its activity as an infecting agent. Such proof must be furnished by the evidence of some definite tissue reaction or the appearance in the blood-serum of agglutinins, protective substances, etc.

*Bacillus coli* is, however, associated with a great variety of lesions in man of which it is evidently the etiological agent. The lesions are most commonly found in the structures in the abdominal cavity. Thus *Bacillus coli* is a frequent isolation in the pus in appendicitis,

often in association with the streptococcus. In many instances it is apparently the only organism present. It is more frequent in those cases with walled-off abscesses containing a fetid, foul-smelling pus than when generalized peritonitis develops. In cases of general peritonitis following perforation of the gut, as in typhoid fever, *Bacillus coli* is nearly always present, usually with other intestinal organisms. It may occur in pure culture in peritonitis without intestinal perforation, as in the case reported by Welch.[33] It is present in inflammatory conditions in the liver and gall-bladder, in liver abscess, in the bile in gall-stones, in acute hemorrhagic peritonitis (Welch). In the genito-urinary apparatus it appears as a frequent cause of cystitis, especially in cystitis with acid urine. It may also be isolated from pyelonephritis. *Bacillus coli* is frequently the only organism to be found in localized abscesses beneath the skin, especially in those with a foul fetid odor. It was early described in pus as *Bacillus pyogenes fetidus* by Passet.[34] Abscesses about the anus and rectum show frequently *Bacillus coli* as the only organism present. It is a frequent cause of wound infections. Levy[35] reported the isolation of *Bacillus coli* fifteen times in operative wounds, laparotomy wounds especially.

*Bacillus coli* is occasionally isolated from the blood during life, the patients presenting a clinical condition like a true septicemia. Thus Hitschmann and Michel[36] reported a septicemia from *Bacillus coli* following catheterization, Stern[37] and Étienne[38] a similar septicemia in which the portal of entry was a diseased gall-bladder. Such septicemias have been especially emphasized by Gilbert[39] as "colibacillosis." In the majority of cases, especially in adults, some definite portal of entry for the organism can be found in pathological conditions associated with the intestinal tract. It is not entirely clear whether such septicemias can occur in adults from invasion of the body from injured intestinal epithelium. In the cases studied by Coleman and Hastings[40] *Bacillus coli* was isolated from the blood-stream in cases resembling typhoid fever, but from whom the typhoid bacillus could not be isolated and in whose blood agglutinins for the typhoid bacillus were not demonstrable. In newborn children such septicemias have been described by Kamen[41] in epidemic form. During life the children show slight jaundice and diarrhea without fever, dying in three to four days. At autopsy they have hemorrhagic infarcts in the lungs, acute splenic tumor, fatty muscles, and interstitial nephritis. This condition is sometimes described as Winckel's disease or "hemorrhagic septicemia" of the newborn. Examples of this condition have been reported from Escherich's clinic by Kowalewsky and Moro.[42] In one instance a virulent strain of *Bacillus coli* was obtained from the urine, and was agglutinated by the patient's blood in a dilution of 1 : 100. At autopsy *Bacillus coli* was isolated from the blood and all the organs. In another case which showed jaundice, splenic tumor, and a bloody diarrhea, *Bacillus coli* was obtained from the blood which showed an agglutination of 1 : 50 to 1 : 100 for the isolated strain. At autopsy *Bacillus coli* was everywhere present. Similar cases were reported earlier by Lubarsch and Tsutsui.[43] By many authors, especially among the French, virulent strains of *Bacillus coli* are assigned an important rôle in diarrhea and dysentery. These cases are explained on the assumption that virulent strains of the organism, or strains foreign to the individual's intestinal flora, are taken in with food or drink. While there is a large amount of evidence that strains from diarrhea or dysentery may be highly virulent for animals, evidence that they play an etiological rôle in the production of disease in man is not entirely satisfactory. The recent investigations of Smith on "scours" in calves, in which foreign strains of *Bacillus coli* play a partial rôle in the production of the condition, are extremely interesting in this connection.

**Serological.**—In animals immunized to *Bacillus coli* agglutinins, bacteriolysins, and complement-fixing substances appear in the blood. Occasionally such substances appear in the blood in man as a result of infections with the organism.

**Distribution.**—*Bacillus coli* is widely distributed. It is the predominant organism of the dejecta of man and of many animals and is always present in the intestinal tract of all species under normal circumstances. It is usually more abundant in the lower portions of the bowel, in the colon, cecum, and rectum, than above in the stomach and small intestine. It occurs almost constantly in areas of the environment exposed to fecal contamination like the soil and water. It is frequently present in cow's milk and in various types of food. Occasionally it is widely distributed in the environment. Thus it may be isolated with considerable frequency from the floors, from the surface of furniture, and from various objects which are constantly handled, in rooms where a number of individuals are gathered together, especially if they are uncleanly in their habits.

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**Varieties of Bacillus coli.**—Different strains of *Bacillus coli* show different reactions for carbohydrates. The most important sugar in this connection is saccharose, as originally pointed out by Durham. According to this author the original strain of *Bacillus coli* obtained by Escherich had no action on saccharose and Durham proposed that the name *Bacillus coli communis* (*Bacterium coli commune*) be limited to this strain. For the saccharose-fermenting variety, which Durham regarded as more common than the other, he proposed the name *Bacillus coli communior*. This variety is now frequently called *Bacillus communior*. The strains of this organism fermenting saccharose do not differ in any other respects from those failing to act on this carbohydrate. The morphology and staining properties, cultural reactions, and pathogenic properties are identical. It is generally found that the saccharose-fermenter is more common than the saccharose-non-fermenter. Thus in 60 strains of *Bacillus coli* from the intestinal contents of calves the present author has found 40 strains which did not ferment saccharose and 20 strains which did act upon this carbohydrate. This is about the proportion established by Durham for man.

The fermentation of other carbohydrates is also somewhat variable. Thus Burke has found a reaction with dulcite in 20 strains out of a total of 22. The non-motile variety of *Bacillus coli* is sometimes called *Bacillus coscoroba*, a name originally proposed by Castellani. As pointed out above the motility of *Bacillus coli* is subject to the greater variation. The same strain may be actively motile at certain times, devoid of motility at other times. A strain called *Bacillus coscoroba*, obtained from the American Museum, was actively motile. It is questionable, therefore, whether the non-motile strains of *Bacillus coli* should be given specific rank.

Castellani: Ann. de l'Inst. Pasteur., 1900, xiv, 224.

### Bacterium Group II

Non-pigmented, non-liquefying organisms, fermenting dextrose and other carbohydrates, but not saccharose or lactose. Gram-negative. Pathogenic to man and to many species of animals. The paratyphoid group.

*Bacillus suipestifer* Salmon and Smith.
" *enteritidis* Gärtner.
" *Aertrycke* De Nobele.
" *nodulificans* Langer.
" *typhi murium* Loeffler.
" *paratyphosus* B Schottmüller.
" *paratyphosus* A (Brion and Kayser. Gwyn).
" *paratyphosus* C Uhlenhuth and Hübener.
" *psittakosis* Nocard.
" *pestis caviæ* Wherry.
" *of calf diarrhea.*
Danysz bacillus of rat plague.
*Bacillus abortus equi* Meyer and Boerner.
*Bacterium anatum* Rettger and Scoville.
Morgan's bacillus No. 1.
Paratyphoid bacillus X of Mita.

**The Paratyphoid Group.**—The first member of the group of organisms, known usually at the present time as the paratyphoid or the intermediate group, was described by Theobold Smith as the "hog-cholera bacillus." Smith pointed out the distinguishing features of this organism and the methods of separating it from *Bacillus coli* by the fermentation of the sugars. His observations were quickly confirmed by other investigators, especially by Welch and by Moore in this country.

Similar organisms were found in other diseases of animals, especially by Gärtner in cattle (*Bacillus enteritidis*), by De Nobele in calves (*Bacillus Aertrycke*), by Loeffler in mice (*Bacillus typhi murium*). Apparently members of the group were first observed in man by Gilbert[1] who called them "paracolon bacilli," his observations being confirmed by Gwyn in America. Achard and Bensaude[2] first made use of the term "paratyphoid," isolating these organisms from 2 cases resembling typhoid fever. A careful study of the different members of the group was made by Cushing, who described it as the hog-cholera, Gärtner or intermediate group. About this time the great importance of the paratyphoid bacilli for man was recognized by Schottmüller,[3] Kurth,[4] Brion and Kayser,[5] and others in Germany. Schottmüller differentiated two types of paratyphoid bacilli by cultural and agglutination reactions, these types being later designated *Bacillus paratyphosus* A and *Bacillus paratyphosus* B. *Bacillus paratyphosus* A, sometimes known as the Brion-Kayser bacillus, plays a relatively unimportant part in the etiology of human infections. *Bacillus paratyphosus* B has been isolated from cases of continued fever all over the world and has been extensively investigated. Much of the disease caused by it resembles typhoid fever clinically. In other instances the paratyphoid infections exhibit symptoms of acute enteritis, traceable to the production of a heat-resistant toxin on foods. The paratyphoid infections in some instances thus become closely similar to the cases of meat poisoning from other members of the group.

The paratyphoid group is large and somewhat diversified, since its representatives have been found in nearly every species of animal. The relation-

ship of the various organisms to each other is very complicated. Important contributions have been made to the subject by Trautmann[6] and Uhlenhuth[7] in Germany and by TenBroeck,[8] Krumwiede,[8] and Jordan[9] in this country.

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### BACILLUS SUIPESTIFER Salmon and Smith

Described originally by Salmon and Smith,[1] as the etiological agent in hog-cholera, a widely disseminated disease in America, England, and on

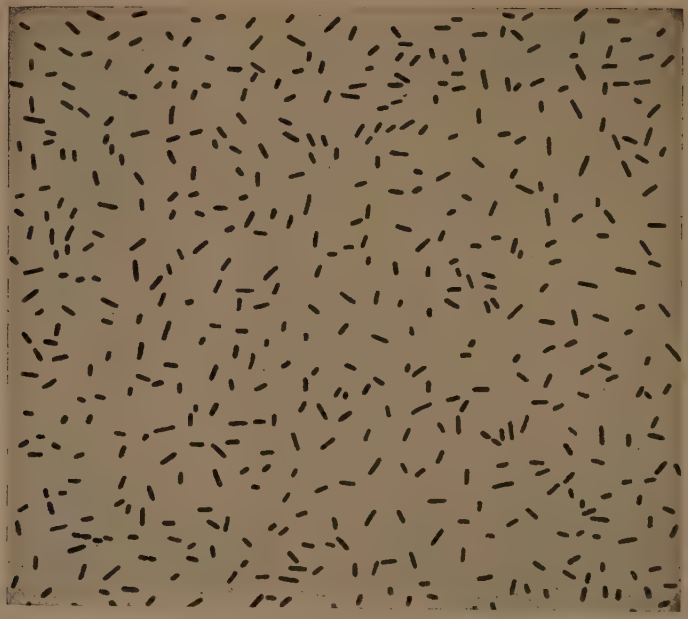

Fig. 76.—*Bacillus suipestifer* from plain agar thirty hours old, stained with gentian-violet.

the continent of Europe. The organism was almost constantly present in the lesions of the disease and produced pathological changes in experimental animals which closely resembled those found in naturally infected swine. Since then the true etiological agent of hog-cholera has been shown to be a filtrable virus and *Bacillus suipestifer* to be a secondary invader. Investigated especially by Smith.[2]

**Morphology.**—Small, rather thin and delicate organisms measuring, from plain agar twenty-four hours old, .3 to .4 $\mu$ in width by 1 to 1.5 $\mu$ in length. Longer forms measuring 2 to 2.5 $\mu$ in length and short almost coccoid organisms are occasionally seen. On dextrose agar the organisms are somewhat enlarged, measuring usually 0.5 $\mu$ in width by 2 to 3.5 $\mu$ in length. No spores.

**Motility** active, due to peritrichic flagella (Moore[3]).

**Staining Reactions.**—Stained easily by gentian-violet, methylene-blue, or dilute carbol-fuchsin. Polar granules show on many organisms, best brought out by dilute carbolfuchsin. Gram-negative.

**Cultivation.**—Cultivated without difficulty on the usual media. Growth best at 37° C., but growth also at low temperatures, 8° C. (Frosch[4]) and at high temperatures 45° C. (Poels[5]) where it loses motility and power of gas production from carbohydrates. Best development on neutral or weakly alkaline media, but growth also on slightly acid media.

**Cultural Characters.**—*Plain Agar Plates.*—Colonies appear on the surface in twenty-four hours, small delicate and translucent, 1 to 2 mm. in size. Under low power granular with dark centers, often nucleated, margins thin, edges entire. Deep colonies punctiform. Under low power usually ovals and disks. The colonies grow slowly, remaining flat and smooth and reaching a diameter of 2 to 4 mm. after five to six days. In older plates, ten to twelve days, the

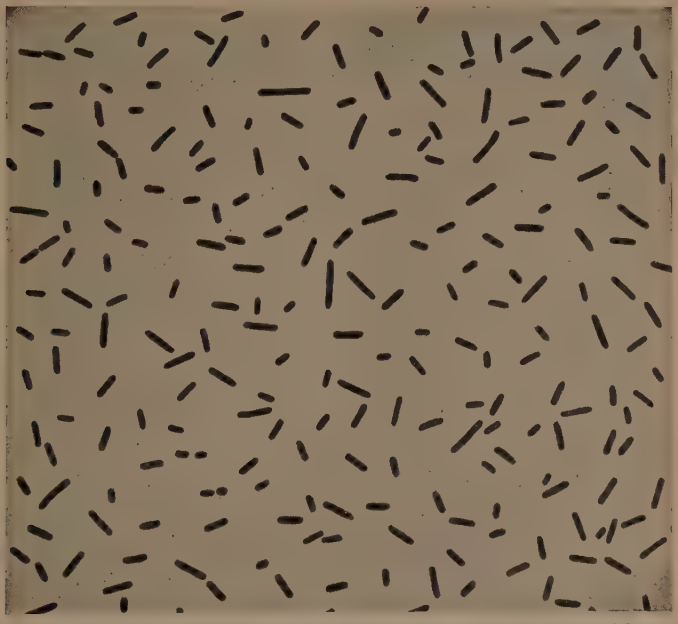

Fig. 77.—*Bacillus suipestifer* from dextrose agar twenty hours old, stained with gentian-violet.

surface colonies are often 4 to 5 mm. in diameter, with thin margins and crenated edges, while the deep colonies are well developed ovals and disks, $\frac{1}{2}$ to 1 mm. in size.

*Plain Agar Slant.*—Moist, thin growth in twenty-four hours spreading especially along the lower part. The growth increases fairly rapidly in extent, and in three to four days is wide spread but thin, and moist. In older cultures ten to twelve days, the growth spreads all over the lower portions of the agar and the water of condensation may be faintly turbid.

*Plain Agar Stab.*—Delicate line and surface growth in twenty-four hours, increased in forty-eight hours. The development remains rather delicate along the line and spreads slightly at the point of puncture.

*Dextrose Litmus Agar Plates.*—Superficial colonies in twenty-four hours delicate, flat, usually round, 1 to 3 mm. in diameter. Under low power thin, somewhat opaque, often striated, margins thin, edges entire. Deep colonies small and punctiform, under low power usually oval or disk-shaped. The agar shows an acid reaction with bubbles of gas, in various areas. The surface colonies increase somewhat in size in three to four days and after ten to twelve days may measure 4 to 5 mm. in diameter, especially if isolated. The deep colonies are round, or oval, $\frac{1}{2}$ to 1 mm. in size. The acid reaction remains permanently.

*Dextrose Litmus Agar Slant.*—Moderate growth in twenty-four hours with an acid reaction

and bubbles of gas, The growth extends over the agar surface in four to five days, remaining flat and smooth, usually whitish. Acid reaction usually permanent.

*Dextrose Litmus Agar Stab.*—Abundant line and surface growth. Agar acid and fragments by bubbles of gas.

*Gelatin Plates.*—Small, delicate, flat, thin colonies, 1 to 2 mm. in size in twenty-four to forty-eight hours. Under low power granular, round or irregular, with leaf-like margins and entire edges. Some colonies distinctly feathery. The colonies increase but slowly in size. Older colonies under low power may be very irregular with outlying strands of growth. No liquefaction.

*Gelatin Stab.*—Delicate line and surface growth in twenty-four to forty-eight hours. The line growth remains delicate even in old cultures while a moderate surface growth develops at the point of puncture. No liquefaction.

*Blood-serum.*—Delicate, smooth, slightly elevated growth in twenty-four hours. The growth increases moderately and at the end of nine to ten days is delicate, often cream-colored, with elevated margins.

*Broth.*—Faint turbidity and crumbly sediment in twenty-four hours with an occasional fragile film. The turbidity remains moderate even in old cultures, the sediment often increased in amount, whitish.

*Peptone.*—Faint turbidity and sediment in twenty-four to forty-eight hours. The turbidity becomes somewhat more marked in old cultures, the sediment whitish or yellow-brown.

*Potato.*—On acid potato a thin white expansion appears in twenty-four to forty-eight hours. On alkaline potato the growth is heavier and lemon yellow in twenty-four to forty-eight hours, later thick, straw-yellow, yellow-brown, or brown. Growth often barely visible (Smith).

*Litmus Milk.*—Abundant development. Faint acidity in twenty-four hours, yielding in two to three days to a neutral or alkaline reaction with an alkaline cream ring. In eight to ten days the milk is distinctly alkaline, blue above, often white below from a reduction of the litmus. In older cultures the milk is usually deep blue. Later the milk may become opalescent and brownish. No coagulation and no digestion of the protein. In old milk cultures the fat is saponified, due possibly to a fat-splitting ferment.

*Petruschky Litmus Whey.*—Slight uniform turbidity and at first an acid reaction with a red color. The reaction changes to alkaline on the third or fourth day and an intense blue color appears. Usually a film develops on the surface. The change from acid to alkali may occur in forty-eight hours with some strains, be delayed ten to twelve days in others.

*Special Media.*—Blue colonies on lactose litmus agar of v. Drigalski and Conradi, center sunken after a few days with wall-like peripheral edges. On Loeffler's malachite green slightly turbid, but transparent colonies, surrounding medium clear and yellow. Colorless colonies on Endo and on Kindberg media.

Methyl red reaction positive.

Voges-Proskauer negative.

**Fermentations.**—In Smith fermentation tubes containing glucose and sediment develop in the bulb in twenty-four hours, the turbidity extending into the closed arm. Considerable gas is evolved, usually a column in the closed arm about 1 inch in height. Reaction acid. About $\frac{1}{4}$ to $\frac{1}{3}$ the gas is absorbed by KOH, the balance explosive. Gas formula, $\frac{H}{CO_2} = \frac{3\text{–}4}{1}$. In saccharose and lactose fermentation tubes the growth stops at the bend of the tube, the broth in the closed arm remaining clear. Reaction alkaline.

Besides glucose the hog-cholera bacillus ferments levulose, galactose, mannose, maltose, rhamnose, mannite, dulcite, sorbite. Besides saccharose and lactose, it fails to ferment raffinose, glycogen, inulin, potato-starch, glycerin, erythrit. There is some difference of opinion in regard to xylose and arabinose. In the author's cultures, American in origin, xylose and arabinose were not attacked.[6] Uhlenhuth, Haendel, and Schirn[7] state that these sugars may be fermented.

**Mutations.**—Baerthlein[8] has described mutations in this organism like those common to other members of this group. He has found large feathery turbid colonies, small light colonies with smooth edges. light transparent colonies, turbid, opaque colonies, and small turbid iridescent colonies like mother-of-pearl. The light colonies consist of delicate, slender bacilli, the turbid of thick plump rods sometimes segregated, and the iridescent colonies of long, slender bacilli.

**Chemical Products.**—Indol is produced by most American strains (Smith and Moore[9]) and by some European strains (Voges-Proskauer[10]). Hydrogen sulphid is produced in peptone solution by all strains.

**Resistance.**—Organisms survive long periods, weeks or months, in media protected from drying. Survive drying several months. Survive in water and upper layers of soil up to four months. Isolated by Koske from the cadaver of a shoat one hundred and sixty days after burial.

Destroyed at 60° C. in ten to twenty minutes (Salmon and Smith). Killed in a short time by ordinary disinfectants.

**Pathogenic** to mice, guinea-pigs, and rabbits by subcutaneous, intravenous, and intraperitoneal inoculation, also by inhalation, according to Frosch. Rats less sensitive. Different strains differ greatly in virulence. With cultures of low virulence subcutaneous injection is followed by a hard infiltration with a tendency to abscess formation. Highly virulent cultures kill mice and guinea-pigs by subcutaneous injection of small doses in twenty-four to forty-eight hours, rabbits in two to five days. Intravenous and intraperitoneal inoculation usually kill rabbits more rapidly. The animals die of septicemia and at autopsy the organisms can be cultivated from the blood, all the organs, and from the intestine. A turbid serous exudate is found in the large body cavities with a fibrinous deposit on the serous membranes, often containing small hemorrhages. Spleen and liver enlarged and show small circumscribed necrotic foci which may resemble the lesions in tuberculosis. Outspoken lesions of the intestines and patches in the lungs resembling croupous pneumonia may also occur. In rabbits characteristic ulcers are found following injection of the organism into the cecum. By infection per os a necrosis develops in the lymphoid follicles of the intestine with subsequent ulceration (Welch and Clement,[11] Karlinsky[12]). Pigeons die from subcutaneous and intramuscular injection in one to three days (Salmon and Smith). Injection into the breast muscle leads to its degeneration and eventually to its complete decay. (Seiffert[13] claims that all the organisms in this group produce this change in pigeon muscle.) Hens and ducks refractory.

With large animals, cattle, goats, asses, and horses subcutaneous injection is followed by large localized abscesses (Salmon and Smith, Joest[14]) and intravenous injection produces a severe general reaction. Calves develop severe enteritis and die from alimentary administration (Uhlenhuth, Hübener). Swine are especially sensitive to the organism. Subcutaneous injection is followed by local abscess formation and later death from septicemia, intravenous injection by septicemia. Administration by mouth is followed by the formation of ulcers, which are practically identical with the so-called buttons found in the natural disease (Welch and Clement). Infection can also be produced by feeding.

The virulence of cultures is retained long periods under artificial cultivation. Thus Salmon and Smith report the survival of organisms one and one-half years in blood-serum stabs without loss of virulence.

Infection from this organism is rare in man. According to Manninger[15] it may occur, however.

**Toxins.**—Old cultures of the hog-cholera bacillus killed by chloroform and filtrates from such cultures are toxic to small animals in small doses (Salmon and Smith, de Schweinitz,[16] Ostertag,[17] Karlinsky). The poisons in such cultures can be heated to 100° C. without destruction. They are possibly intracellular poisons liberated on the death of the organism. Great variation is seen in the capacity of different strains to produce these bodies, the filtrates from young cultures not being toxic.

**Distribution.**—Hog-cholera bacilli are present in the organs in many cases of natural hog-cholera and have been found in the intestines in healthy swine in a small proportion, 8.4 per cent. in 600 cases (Uhlenhuth and Haendel[18]). From this area they invade the body in hog-cholera.

**Varieties of Bacillus Suipestifer.**—Seven different varieties or strains of the hog-cholera bacillus were isolated by Smith, differing somewhat in cultural characters and virulence. One of these isolated by Moore was non-motile and a similar non-motile strain was obtained by Smith.[19] Strains are fairly common which do not respond to serological tests. Some of these on artificial cultivation become agglutinable by *Bacillus suipestifer* serum, while others fail to assume this character. Dorset[20] has found a strain which fails to produce gas in dextrose, but is otherwise typical. Two aberrant strains have been especially studied in Europe, *Bacillus typhi suis* of Glaser[21] and *Bacillus suipestifer* Voldagsen of Dammon and Stedefeder.[22] Uhlenhuth and Haendel regard these organisms are identical. In morphological and cultural characters they do not differ essentially from *Bacillus suipestifer*. They have a pathogenic action for small animals like that of the hog-cholera bacillus, but are much less virulent. They do not form a soluble toxin. They are especially virulent to young swine, producing infection by subcutaneous intravenous and alimentary administration. When first isolated they showed but little serological relation to *Bacillus suipestifer*. After long-continued cultivation in the laboratory and passage through animals, their serological reactions changed, and eventually the strains appeared to be clearly related to this organism (Uhlenhuth and Haendel).

1. Salmon and Smith: Am. Month. Micr. J., 1886. Ann. Rep. Bureau of Animal Industry, 1889–1890.
2. Smith: Ann. Rep. Bureau of Animal Industry, 1889–1892. Centralbl. f. Bakteriol. [etc.], 1891, 253; 307; 339.
   Smith and Moore: Centralbl. f. Bakteriol. [etc.], 1894, xvi, 231.
3. Moore: Wilder Quarter Century Book.
4. Frosch: Ztschr. f. Hyg., 1890, ix, 235.
5. Poels: De Varkensziekten in Nederland's Gravenhage, 1905.
6. Ford: Med. News, 1905, lxxxvi, 1127.

7. Uhlenhuth, Haendel, and Schirn: Berl. tierärztl. Wchnschr., 1909, No. 28 and 50.
8. Baerthlein: Arb. a. d. K. Gsndhtsamte, 1912, xl, 433.
9. Smith and Moore: Additional Investigations concerning infectious swine disease. Washington, 1904.
———: Smith: J. Exper. M., 1897, ii, 543.
10. Voges and Proskauer: Ztschr. f. Hyg., 1898, xxviii, 20.
11. Welch and Clement: Internat. Vet. Congress, Chicago, 1893.
———: Welch: Johns Hopkins Hosp. Bull., 1889, i, 9.
12. Karlinsky: Ztschr. f. Hyg., 1898, xxviii, 373.
13. Seiffert: Ztschr. f. Hyg., 1909, lxiii, 273.
14. Joest: Ztschr. f. Fleisch-u. Milchhyg., 1905.
15. Manninger: Centralbl. f. Bakteriol. [etc.], 1924, xciii, 371.
16. de Schweinitz: Phil. Med. News, 1890. Ann. Rep. Bureau of Animal Industry, 1898, xv.
17. Ostertag: Deutsche tierärztl. Wchnschr., 1903–04 etc.
18. Uhlenhuth and Haendel: Schweinepest and Schweineseuche. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, vi, 325.
19. Smith: Centralb. f. Bakteriol. [etc.], 1899, xxv, 241.
20. Dorset: Bureau of Animal Industry, 18th Ann. Rep., 1902.
21. Glaser: Deutsche tierärztl. Wchnschr., 1907.
22. Dammon and Stedefeder: Arch. f. wissensch. u. prakt. Tierh., 1910, xxxvi.

### BACILLUS ENTERITIDIS Gärtner

Discovered by Gärtner[1] in the gastro-intestinal form of meat poisoning from the use of infected meat from cattle.

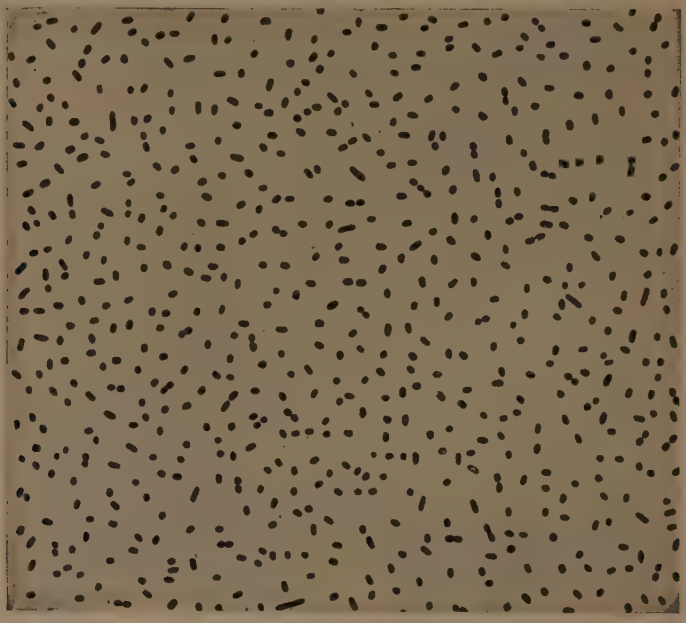

Fig. 78.—*Bacillus enteritidis* from plain agar twenty-four hours old, stained with gentian-violet.

**Morphology.**—Small bacilli measuring 0.3 to 0.4 $\mu$ in diameter and 1 to 2 $\mu$ in length on plain agar, twenty-four hours old, together with many short, almost coccid organisms. Large forms not uncommon, measuring 0.6 to 0.7 by 3 to 4 $\mu$. Slight enlargement on dextrose agar. Long threads rarely seen. Motility active. No spores.

**Stains** by the usual anilin dyes. Gram-negative. Flagella peritrichous.

Aumann[2] reports some strains when first isolated as non-motile, motility appearing on cultivation.

**Cultural characters** like those of the other members of this group.

On *plain agar* the surface colonies are small and delicate, measuring $\frac{1}{2}$ to 2 mm. in twenty-four hours. Under low power the colonies are often nucleated, with thin margins and entire

edges. Older colonies may be much larger, ½ to 1 cm. in size when isolated, thicker in the center, margins thin, edges crenated. The deep colonies may also be much more distinct and larger, measuring ½ to 2 mm. in size. On plain agar slants the growth is smooth, rather abundant, often with elevated edges. In stabs the line and surface development are also fairly vigorous.

*Dextrose litmus agar colonies* small and delicate in twenty-four hours, 1 to 2 mm. in size, older colonies 4 to 5 mm. Deep colonies well-marked and distinct, about ½ mm. in size. Agar highly acid with bubbles or blebs of gas. Acidity permanent. Fairly abundant growth in dextrose litmus agar slant and stab with acidity and gas. Acidity permanent.

*Gelatin* colonies small and delicate in forty-eight hours, ½ mm. in size, under low power either round, regular and entire, or distinctly feathery. Some colonies under low power appear moruloid, with petal-like margins. Older colonies may be somewhat larger, 1 mm. in size, and under low power show dark nuclei with finger-like prolongations. Delicate line and surface growth in gelatin stabs. No liquefaction.

Turbidity and fairly heavy whitish sediment in *broth* and *peptone*. Yellowish or yellowish-brown growth on *alkaline potato*. Cream-colored growth on *blood-serum*.

Fig. 79.—*Bacillus enteritidis* from dextrose agar twenty-four hours old, stained with gentian-violet.

*Litmus milk* shows an acid reaction in twenty-four hours with a decolorization of the litmus below. In three to four days the acidity gives way to an alkaline reaction and gradually the milk turns deep blue. In old cultures the fluid may be opalescent, brownish, and highly alkaline. No coagulation.

*Petruschky's Litmus Whey.*—Some strains show the typical reaction, acidity at first followed by an alkaline reaction in four to six days. Other strains revert to alkali at once and still others produce so little alkali that the end reaction is only violet.

Methyl red positive.

Voges-Proskauer negative.

No hemolysis on blood agar plates.

Produces hydrogen sulphid. No indol. No creatinin.

Nitrates reduced to nitrites.

**Fermentation Reactions.**—In dextrose fermentation tubes turbidity and sediment develop in the bulb, the turbidity extending into the closed arm with an evolution of gas with an acid reaction. About 1 to 1¼ inch of gas collects in the arm, of this one-fifth to one-fourth is absorbed by KOH, balance explosive $\left(\frac{H}{CO_2} = \frac{3\text{–}4}{1}\right)$. In saccharose and lactose tubes the growth is limited to the bulb, the arm remains clear and the reaction is alkaline.

Besides dextrose the Gärtner bacillus ferments with acid and gas, levulose, galactose,

mannose, maltose, rhamnose, arabinose, xylose, dulcite, and mannite. It usually fails to attack saccharose, lactose, melitose, raffinose, melifiose, erythrit, starch, dextrin and inulin.

**Resistance and viability** fairly high. Survives three years in fused agar tubes (Martini). Found by v. Drigalski[3] in the cadaver of a horse three years after burial. Obtained by Kathe[4] from the body of a child fourteen days after death. Destroyed by heat at 55° to 60° C. in twenty to thirty minutes. In cultures destroyed easily by the usual disinfectants. According to Bernstein the Gärtner bacillus can develop in meat preserved in boric acid. Klein found an abundant growth in calf and swine meat infusions containing 0.5 per cent. boric acid. Other preservatives have no definite action on the organism in meat except in high concentrations.

*Pathogenic* to small animals (mice, guinea-pigs, and rabbits), different strains differing considerably in virulence. The animals die of a septicemia and at autopsy the organisms may be isolated from the blood and the organs. The lesions resemble those found with the hog-cholera bacillus, but are not so marked. Occasionally small focal necroses are present in the liver. Gärtner's original strain was pathogenic to pigeons, sheep. and goats also, but not to cats, sparrows, and hens. The animals developed an intoxication and septicemia from inoculation, an enteritis and lesions of the nervous system from feeding of killed cultures. The Moorseler bacillus isolated by van Ermengem,[5] closely related if not identical with the Gärtner bacillus, produced a severe enteritis in calves from inoculation or from feeding. The cooked meat containing the organism produced an acute enteritis when fed to apes.

Injection of Gärtner bacilli into the udder of goats is followed by septicemia (Reinhardt and Seibold[6]), into the udder of cows by purulent inflammation. Uhlenhuth and Haendel[7] claim that injections of Gärtner bacilli produce in swine the lesions characteristic of hog-cholera.

**Toxins.**—The Gärtner bacillus produces a soluble poison which may be found in filtrates from old cultures (Gärtner, Kruse,[8] and van Ermengem). The poison from van Ermengem's strain (Moorseler bacillus) withstood a temperature of 100° to 120° C. In v. Drigalski's strain (Neunkirchner) twelve-day cultures could be heated to 70° C. three hours or boiled ten minutes without losing toxicity; 0.3 to 0.4 c.c. injected subcutaneously killing mice and guinea-pigs. The filtrate killed guinea-pigs in 4 c.c. quantities, but not pigeons. Rimpau[9] found poisons in filtrates from broth cultures ten days old, which after heating killed mice, guinea-pigs, and rabbits. The poisons lose their activity rapidly (Gärtner).

Agglutinins are produced easily in animals by injection of the Gärtner bacillus. They reveal some considerable difference in the strains and the grouping at best is unsatisfactory. According to Aumann freshly isolated strains are not agglutinated but become agglutinable after laboratory cultivation. The organism is also agglutinated at times by *Bacillus typhosus serum* and the typhoid bacillus is occasionally agglutinated by Gärtner serum.

**Distribution in Man.**—In Gärtner bacillus infections in man, the organisms are found in the stools and in the blood-stream. Agglutinins appear in the patient's blood but disappear rapidly (three weeks). Chronic carriers apparently do not develop. Cholecystitis with gallstones has been reported by Dean.[10] According to Rimpau Gärtner bacilli have been found in the stools in individuals who have partaken of infected meats and yet developed no symptoms. Contact infection is rare, but has been reported in Germany. The diagnosis in man can be established only by the isolation of the organism.

**Occurrence.**—In many localities the Gärtner bacillus is isolated from cases of meat poisoning more often than any other organism, as in Hamburg (Aumann). It is seldom isolated except from meat. It has been found however in chocolate pudding by Aumann and recently in oatmeal by Krumwiede. According to Uhlenhuth it is found as a secondary invader in swine dead of natural hog-cholera.

1. Gärtner: Bresl. ärztl. Ztg., 1888.
2. Aumann: Centralbl. f. Bakteriol. [etc.], Orig. 1911, lvii. *Ibid.*, 1912, lxiii.
3. v. Drigalski: Festschr. z. 60. Geburts. v. Robert Koch, Jena, 1903.
4. Kathe: Med. Klin., 1910, vi, 1, 907.
5. van Ermengem: Bull. Acad. roy. de méd. de Belg., 1892. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 1903, ii, 637.
6. Reinhardt and Seibold: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxvi, Orig., 159.
7. Uhlenhuth and Haendel: Schweinepest and Schweineseuche. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, i, 325.
8. Kruse: Allgemeine Mikrobiologie. Leipzig, 1910.
9. Rimpau: Arb. a. d. K. Gsndhtsamte, 1909, xxx, 330.
10. Dean: J. Hyg., 1911, xi, 259.

### Bacillus Aertrycke De Nobele

Found originally by De Nobele[1] in an epidemic of food poisoning at Aertrycke, Belgium, involving about 50 individuals who became seriously ill after eating veal from calves suffering from a chronic enteritis. The

symptoms consisted of vomiting, fetid diarrhea, pains in the head, intense thirst, etc. Only one person died, the others recovering after a long period of illness. Two dogs and a cat which ate the meat also became ill with vomiting and diarrhea. From portions of the flesh and bone of the animal, mice, guinea-pigs, and rabbits were inoculated subcutaneously, all dying within two to five days. Pure cultures of an organism were obtained from the subcutaneous exudate, blood, liver, and spleen, which resembled the organisms obtained by van Ermengem from sick calves at Moorseele.

**Morphology.**—Bacteria with round ends, often in pairs, 0.6 to 0.8 $\mu$ in length, decolorizing by Gram. In young cultures and in the juice of organs the central areas stain less intensely than the extremities.

**Motility** active, due to numerous short cilia.

**Cultivated** easily on ordinary media, best at 37° C. Abundant gas on glucose agar. Milk not coagulated after several weeks. It loses its capacity and becomes highly alkaline. Turbidity rapidly formed in alkaline bouillon with a surface pellicle easily dislodged. Odor not putrid or foul. Indol not produced, even after ten days. Grayish non-elevated growth on potato.

**Virulent** to mice, guinea-pigs, and rabbits, doses of 0.1 c.c. killing them generally in three to six days. At autopsy there is suppuration and necrosis of the subcutaneous tissues, congestion of the intestine, hyperemia of the liver, acute splenic tumor, etc.

The organism was agglutinated by the blood-serum of the patients suffering from the food poisoning. The agglutinins were present in a dilution of 1 : 50 and in one instance in a dilution of 1 : 400.

**Remarks.**—A number of other organisms identified as *Bacillus Aertrycke* have been obtained by other observers. Thus Durham[2] isolated a bacillus from the liver of an individual who had died of food poisoning and subsequently studied an epidemic involving 185 individuals at Chadderton (Oldham) who had eaten veal pâté. The blood of 19 of these cases agglutinated the bacillus previously found. The agglutination ran as high as 1 : 1000 in certain individuals and in 5 persons *Bacillus typhosus* was agglutinated in a dilution of 1 : 100. Schütze obtained an organism from an epidemic at Newport and this is known as the Newport strain. Hutchins isolated one from an epidemic at Newcastle known as the Mutton strain. According to Bainbridge *Bacillus Aertrycke* is indentical with *Bacillus suipestifer*. In this country the organism is sometimes regarded as the same as *Bacillus pestis caviæ* of Smith (Krumwiede). The British Medical Research Committee[3] have reported an epidemic caused by *Bacillus Aertrycke*, the strain found (Aubengue strain) being identical with the Newport strain. According to this committee *Bacillus Aertrycke* ferments with acid and gas, glucose, levulose, galactose, maltose, arabinose, mannite, dulcite, and sorbite, and does not ferment lactose, saccharose, raffinose, salicin, and inulin. Litmus milk is rendered first acid and then alkaline. In general British workers differentiate *Bacillus enteritidis* of Gärtner from *Bacillus Aertrycke*, *Bacillus paratyphosus* B and *Bacillus suipestifer*, the last three being indistinguishable by agglutination. *Bacillus paratyphosus* B may be differentiated by absorption of agglutinins from *Bacillus suipestifer* and *Bacillus Aertrycke* which are identical by absorption.

Bergey[4] reports the fermentation of isodulcite and inosite in addition to the other carbohydrates mentioned, with acid production in glycerol.

1. De Nobele: Ann. Soc. de méd. de Gand., 1898, lxxvii, 281.
2. Durham: Brit. M. J., 1898, ii, 1797.
3. Medical Research Committee Special Report Series, No. 24.
4. Bergey: p. 214.

### BACILLUS NODULIFICANS Langer

A number of paratyphoid or Gärtner bacteria were described in calves by Langer[1] as the cause of the special disease of the liver characterized by the formation of grayish, submiliary, necrotic nodules in this organ. From these foci Langer obtained an organism which he described as a new species. Subsequently Pitt[2] showed that Langer's organism is related serologically to the Gärtner bacillus. Francke[3] reports that *Bacillus coli* can produce similar nodules in the liver.

**Morphology,** cultural reactions, and pathogenicity the same as with the other members of this group.

1. Langer: Ztschr. f. Infektionskr. d. Haustiere, 1904.
2. Pitt: Centralbl. f. Bakt., [etc.], 1909, xlix, 593.
3. Francke: Ztschr. f. Fleisch-u. Milchhyg., 1909, H. 3.

### BACILLUS TYPHI MURIUM Loeffler

Described by Loeffler,[1] in 1890, in an epizoötic among mice called mouse typhoid, and recognized as a member of the same group with the hog-cholera bacillus and the Gärtner bacillus by Smith.[2] First found in man by Trommsdorff[3] in 1903 in cases of gastro-enteritis. Formerly, much used as a virus for the destruction of mice.

**Morphology and Cultural reactions** like those of the other paratyphoid bacilli. On *plain agar* in twenty-four hours the organisms are small, measuring usually 0.3 to 0.4 $\mu$ by 0.7 to 1.5 $\mu$, with numerous short forms. Slightly enlarged on dextrose agar. Actively motile. Peritrichous flagella. Gram-negative. *Agar colonies* small, flat, 2 to 4 mm. in twenty-four hours. Rather delicate, smooth, moist growth on *plain agar*, often with crenated edges. Flat smooth colonies on *dextrose litmus agar*, 1 to 3 mm. in diameter, in twenty-four hours. Agar acid with gas bubbles or blebs. Acidity permanent. *Dextrose litmus agar slant* shows smooth moist growth with acidity and gas formation. Gelatin colonies small and delicate in forty-eight hours, often with fringed margins. Under low power they show dark centers with feathery peripheries. Other colonies round and regular or moruloid with petal-like extensions. Delicate growth in *gelatin stab*. No liquefaction of *gelatin*. Turbidity and sediment in *broth* and *peptone*, without scum. Little or no growth on *potato*. Delicate cream-colored growth on *blood-serum*. *Milk* at first faintly acid, then alkaline, usually in four to five days. Old milk tubes become deep blue. Ferments dextrose with acidity and gas. Gas formula, $\frac{3-4}{1}$. Fermentation also of galactose, levulose, mannose, rhamnose, maltose, arabinose, mannite, dulcite. No fermentation of lactose, saccharose, melitose, raffinose, melibiose, xylose, erythrite, starch, dextrin, or inulin nitrates not reduced to nitrites.

**Pathogenic** to laboratory animals in much the same fashion as the other members of this group, but intensely virulent to mice. With his culture Loeffler infected field mice by feeding, the animals dying in eight to twelve days. Infection by mouth not produced in field mice, rats, guinea-pigs, rabbits, pigeons, and hens. Virulent to rats, guinea-pigs, small birds, and pigeons by subcutaneous inoculation. Spontaneous infection does not occur apparently with domestic animals.

*Bacillus typhi murium* produces heat-resistant poisons (Bonhoff[4]). Filtrates from broth cultures forty-eight hours old and heated to 100° C. five minutes kill mice in twenty to thirty-six hours in doses of 1 c.c., 0.5 c.c., and 0.1 c.c. injected intraperitoneally. Loeffler found that 0.0001 to 0.01 gram of an agar culture heated two hours to 120° C. or one-half hour to 150° C. killed mice by subcutaneous inoculation.

In Trommsdorff's human cases the patients become infected by handling mouse typhoid virus in use for killing mice. Shibayama[5] reported 30 cases of food poisoning from the same organism with two fatalities. These followed the consumption of vegetables prepared in a wooden vessel previously used for mouse typhoid material. He also reported 34 cases with 1 death from eating the flesh of a horse which had recovered from an injection of the organism. The bacteria were found in the horse meat and in the dejecta of the patients. Fleischanderl reported similar cases from handling the virus. Meyer[6] became infected while investigating the organism and cultivated them from his dejecta.

The organism described by Laser[7] from a spontaneous epidemic among field-mice kept in captivity and named *Bacillus muripestifer* by Kruse[8] may be closely related to Loeffler's organism.

1. Loeffler: Centralbl. f. Bakteriol. [etc.], 1892, xi, 129. *Ibid.*. 1893, xiii, 647.
2. Smith, Th.: Ztschr. f. Hyg., 1891, x, 480.
3. Trommsdorff: Arch. f. Hyg., 1906, lv, 279.
4. Bonhoff: Arch. f. Hyg., 1904, l, 222.
5. Shibayama: München. med. Wchnschr., 1907, liv, i, 977.
6. Meyer: München. med. Wchnschr., 1905, lii, 2, 2261.
7. Laser: Centralbl. f. Bakteriol. [etc.], 1892, xi, 184.
8. Kruse: *In:* Die Mikroorganismen (Flügge). 1896, ii, 432.

### BACILLUS PARATYPHOSUS B Schottmüller

First clearly established by Schottmüller[1] as the cause of a disease in man like typhoid fever. Many different strains have been isolated from various parts of the world. These strains have in general the following characteristics, which apply especially to American strains.

**Morphology.**—Small thin organisms measuring 0.3 to 0.4 $\mu$ in breadth and 1 to 2 $\mu$ in length on plain agar twenty-four hours old. Slightly enlarged on dextrose agar. Long threads

rarely seen. *Stains* readily with ordinary anilin dyes. Gram-negative. In films and sections from infected organs polar granules are frequently present.

**Motility** active, somewhat like that of the vibrios. Flagella peritrichic, more numerous than on *Bacillus typhosus*, sometimes longer, frequently curved, forming a kind of network about the organism. Long threads usually non-motile. No motility in cultures kept at 44° to 45° C.

**Cultivated** easily, growing best at 37° C., somewhat more slowly at room temperature. Some growth at 44° to 45° C. (Uhlenhuth and Hübener[2]). Best development on slightly alkaline media, somewhat more abundant than that of *Bacillus typhosus*, less than that of *Bacillus coli*.

**Cultural Characters.**—*Plain Agar Plates.*—Surface colonies in twenty-four hours fairly large, 2 to 4 $\mu$ in diameter, round, regular, flat. Under low power granular, with dark central nuclei and thin margins, edges entire. Deep colonies punctiform, but well-developed; under low power usually oval or disk-shaped, often coarsely granular. Older colonies, three to four days, are larger, especially when isolated, measuring 2 to 4 mm., smooth and glistening. Colonies ten to twelve days old may be $\frac{3}{4}$ to 1 cm. in diameter, often distinctly crenated or furrowed. Deep colonies when older are distinct ovals or disks to the naked eye, $\frac{1}{2}$ to 1 mm. in diameter. Baerthlein[3] reports that paratyphoid bacilli may produce two different types of colonies, large turbid colonies feathery and leaf-like, small homogeneous round colonies somewhat iridescent. Films from the feathery colonies show long threads, from the homogeneous colonies, rods. The colonies occasionally show viscid zones (v. Drigalski,[4] Fischer[5]), especially if kept a day in the thermostat, and then at room temperature when the colonies look like simple viscid drops. Films from such colonies may show organisms with swollen thickenings which take the stain badly (Stromberg[6]).

*Plain Agar Slant.*—Moist, smooth, fairly abundant growth in twenty-four hours, increased in three to four days. By the end of ten to twelve days it is quite abundant, flat, with crenated edges. Water of condensation usually clear.

*Plain Agar Stab.*—Faint line and fairly heavy surface growth in twenty-four hours. The line growth increases in intensity and the surface growth spreads, often widely, with crenated edges. Rarely in old cultures the surface growth reaches the wall of the tube.

*Dextrose Litmus Agar Plates.*—Surface colonies grow widely, measuring 4 to 5 mm. on the surface in twenty-four to forty-eight hours, especially if isolated, They are flat, often crenated, with well-marked striations. Under low power they are thin with striated margins, edges entire. Closely crowded colonies are often only $\frac{1}{2}$ to 1 mm. in size, flat, and regular; under low power smooth, but showing the same striations. Deep colonies small and pin-point. Under low power usually ovals or disks. Agar acid with bubbles of gas. Older surface colonies four to five days are larger, 4 to 5 mm. in diameter, edges elevated and crenated. Deep colonies more marked, oval or wedge-shaped, often $\frac{1}{2}$ to 1 mm. in size. In plates ten to twelve days old the surface colonies are usually 5 to 6 mm. in diameter. Acidity permanent.

*Dextrose Litmus Agar Slant.*—Moderate smooth elevated growth in twenty-four to forty-eight hours, agar highly acid with an occasional bubble of gas. The growth remains moderate in extent even in cultures ten to twelve days old. Acidity permanent.

No hemolysis on blood agar plates.

*Gelatin Plates.*—Colonies appear in two to three days, $\frac{1}{2}$ mm. in size, pale and delicate. Under low power they are often darker in the center with straggling or feathery peripheries Other colonies may be round or have slightly irregular edges. The colonies increase somewhat in size and at the end of eight to ten days are usually only 1 to $1\frac{1}{2}$ mm. in diameter. Often the original colonies show zones of daughter colonies about them. No liquefaction.

*Gelatin Stab.*—Slight line and surface growth in forty-eight hours. The growth increases but slightly, and at the end of ten days is still delicate. No liquefaction.

*Blood-serum.*—Moderate, slightly elevated, cream-colored expansion in twenty-four hours. The expansion increases slightly in three to four days. At the end of ten days it is still moderate, usually cream-colored.

*Potato.*—Moderate yellowish or pale brown growth in two to three days. The growth increases slightly and at the end of eight to ten days is still moderate, usually yellowish or pale brown.

*Broth.*—Faint turbidity and whitish sediment in twenty-four hours, no scum. Turbidity and sediment increase somewhat in three to four days. Later there is a tendency for the broth to clear, the sediment becoming denser, usually whitish. Usually non-viscid on shaking.

*Peptone.*—Turbidity and rather dense flocculent sediment in twenty-four hours, no scum. The turbidity and sediment increase in three to four days, after which the peptone clears slightly, the sediment becoming denser. Non-viscid.

*Litmus Milk.*—Slight acidity in twenty-four hours, yielding to an alkaline reaction in two to three days. The litmus in the depths of the milk is reduced. The alkaline reaction becomes more marked in the milk, with an alkaline cream ring on the surface. In old cultures, ten to twelve days, the milk is deep blue above, often white in the depths. Old milk is syrupy, with the fat saponified.

*Petruschky Litmus Milk-whey.*—Turbidity with a red-violet color due to acid, yielding

to an alkaline reaction and a deep violet-blue color. The change in reaction may take place in twenty-four hours, three to four days, or in eight days. A scum may be formed at the same time.

*Carbohydrates.*—In dextrose fermentation tubes turbidity and flocculent sediment develop in the bulb usually in twenty-four hours. The turbidity extends into the closed arm with an evolution of gas and an acid reaction. About 1 inch of gas collects in the closed arm, of this one-quarter to one-third absorbed by KOH, the balance explosive. Gas formula $\frac{H}{CO_2} = \frac{3-4}{1}$. In lactose and saccharose fermentation tubes the growth is limited to the bulb, the arm remaining clear, the reaction alkaline. Lactose and saccharose not fermented.

Besides dextrose these parathyroid bacilli usually ferment with gas and acid, levulose, galactose, mannose, maltose, xylose, arabinose, rhamnose, dulcite, mannite, and sorbite. They usually fail to attack, besides lactose and saccharose, raffinose, dextrin, inulin, starch, glycogen, glycerin, erythrite.

*Special Media.*—Deep blue colonies on v. Drigalski-Conradi lactose-litmus agar with sunken centers and wall-like edges. White colonies on Endo-agar. Well-developed, glassy, transparent, slightly turbid colonies on Loeffler's malachite green, the green turned to a bright yellow in the vicinity. Large yellow-green transparent colonies on Conradi's brilliant green medium.

Methyl red positive.

Voges-Proskauer negative.

No indol. Nitrates reduced to nitrites. No creatinin. Hydrogen sulphid produced.

**Resistance.**—Paratyphoid B is usually regarded as more resistant than the typhoid bacillus. It survives in the environment, soil, water, etc., for some time. It may survive in the feces two years (Hilgemann, Meyer). In cultures it survives a long time, five months in sealed agar (Martini). Dried on silk threads it may be alive after two hundred and thirteen days (Heim). Lives in milk two to four months, depending on the temperature (Karsten).

Destroyed in broth and milk in one hour at 60° C., in a few minutes at 80° to 100° C. Killed by 1 per cent. formalin in forty minutes. High concentrations of salt (NaCl), 10 per cent. or more, kill paratyphoid bacilli in a short time (Uhlenhuth and Hübener). In meat infected with paratyphoid bacilli and put in brine, the organisms live eighty days with a concentration of 10 to 13 per cent., but do not survive more than forty-five days with a higher concentration.

**Pathogenic Action.**—Paratyphoid bacilli are highly virulent for small laboratory animals (mice, guinea-pigs, and rabbits), but there is great difference in virulence in different strains. With subcutaneous inoculation an induration develops which may lead to abscess formation but the animals are apt to die of septicemia before abscesses form. At autopsy a serous or serofibrinous exudate is present in the body cavities with punctiform hemorrhages on the serous surfaces, The organs show parenchymatous degeneration, the liver multiple areas of focal necrosis, the swollen spleen a few such areas. Frequently a cholecystitis is present (Fraenkel, Much, and Starke[7]). In guinea-pigs subcutaneous injection in doses of 0.05 c.c. of a twenty-four-hour broth culture kills in twenty-four to thirty-six hours. At autopsy, in addition to the changes in the other organs, the adrenals show an intense brown pigmentation like that seen in animals dead of a subcutaneous injection of diphtheria toxin (Bonhoff[8]). The mucous membranes are swollen and reddened with minute hemorrhages. Intravenous injections produce a rapidly fatal septicemia. Intraperitoneal injection of a small portion of an agar culture twenty-four hours old suffices to kill guinea pigs in a few days from seropurulent peritonitis. Mice and rabbits are almost equally sensitive. With rabbits small ulcers are sometimes found in the mucous membranes of the alimentary canal.

Feeding the organisms to animals sometimes leads to infection. Mice are most sensitive to this type of inoculation, then guinea-pigs and rabbits. Rats are refractory. Young animals are more sensitive than old, young cultures more effective than old cultures.

Pigeons are sensitive to intramuscular injection. The introduction of 1/20 to 1/5 c.c. of a twenty-four-hour broth culture into the breast muscle is followed by a rapid destruction of the muscle substance, the animals dying of cachexia in fourteen to twenty days. The remnants of the muscles show miliary gray knots like tubercles (Seiffert[9]), which are not produced by paratyphosus A. According to Reinhold paratyphoid B produces infections in pigeons, ducks, geese, and hens.

**Toxins.**—Killed cultures of *Bacillus paratyphosus* B are poisonous as was first shown by Schottmüller, Brion and Kayser[10] and by Rolly. Filtrates of old cultures are also toxic, 0.1 c.c. of the filtrate of a five-day broth culture killing mice in ten to twenty hours by intraperitoneal injection; 0.5 c.c. killing guinea-pigs (Konrich). Similar toxins were found by Glaser[11] and by Hoffmann.[12] These toxic substances in old cultures are heat-resistant and are usually virulent to laboratory animals by subcutaneous, intraperitoneal, and intravenous inoculation. They are produced inconstantly. Toxic substances have also been found in young cultures by Kraus and Stenizer,[13] like those found in cultures of *Bacillus typhosus*, *Bacillus typhi murium*, and *Bacillus suisepticus*. They killed rabbits by intravenous injection of

1 to 3 c.c. in five to twenty-four hours. Results with mice and guinea-pigs are not so constant. These toxic substances are very labile and are destroyed in a few days.

**Distribution.**—In paratyphoid fever the organisms are present in the blood and in the stools during the course of the disease and also in the various complications, such as cystitis, pyelitis, bronchitis, and pleurisy. They may produce local inflammation and suppuration, arthritis, abscesses in the joints (Achard and Bensaude), myositis, phlebitis (Johnston,[14] Pratt[15]), orchitis, lung abscesses, otitis media, periostitis (Fischer), osteomyelitis (Cushing[16]). Gall-bladder infections are relatively common. At autopsy the bacilli are often widely distributed in the blood and organs. Chronic carriers occur, but more rarely than in typhoid fever (Uhlenhuth and Hübener). They are usually traced to gall-bladder infections. The disease is spread by water, milk, cheese, foods, salads, preserves, and fish.

Various immune bodies develop in the serum of patients with paratyphoid fever, agglutinins, bacteriolysins, complement-fixing bodies, precipitins, etc., but the diagnosis of the disease rests substantially upon the isolation of the organisms from the stools or preferably from the blood. Similar substances are developed in the blood of immunized animals. Agglutination has been largely used in the classification of the strains but the results are very difficult to interpret.

According to Uhlenhuth and Hübener, the only reaction which can be used to separate the Paratyphoids from the Gärtner group is bacteriolysis. A serum made from human paratyphoid dissolves, in the peritoneal cavity of a guinea-pig, human paratyphoids, also mouse typhoid, swine-pest, psittacosis, and calf diarrhea, *but not Gärtner*. A serum made from Gärtner dissolves *Gärtner*, but not paratyphoids.

**Meat-poisoning Type of Paratyphoid B.**—Uhlenhuth and Hübener report that paratyphoid bacilli are frequently the cause of typical meat poisoning. The organisms cannot be differentiated from the organisms isolated from paratyphoid fever in man. They manifest the same resistance to heat and disinfectants, etc. They also produce heat-resistant soluble poisons in culture media. They come from animals infected intra vitam. Clinically these cases present the picture of an intoxication with acute gastro-enteric symptoms as distinguished from paratyphoid fever which is an infection and appears clinically as a subacute typhoid fever. They may also show cholera-like symptoms as in cholera nostras.

Schottmüller believes that paratyphoid infections can be classified as:

1. Paratyphus abdominalis.
2. Gastro-enteritis paratyphosa or cholera nostras paratyphosa.
3. Pyelitis, cholecystitis, endometritis, meningitis, etc., paratyphosa.

1. Schottmüller: Deutsche med. Wchnschr., 1900, xxvi, 511.
———: Ztschr. f. Hyg., 1901, xxxvi, 368.
2. Uhlenhuth and Hübener: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, iii, 1005.
3. Baerthlein: Berl. klin. Wchnschr., 1911, xlviii, i, 373.
4. v. Drigalski: Festschr. z. 60. Geburts. Robert Koch, Jena, 1903.
5. Fischer: Klin. Jahrb., 1906, 61.
———: Ztschr. f. Hyg., 1902, xxxix, 447.
6. Stromberg: Centrabl. f. Bakteriol. [etc.], 1911, lviii, 401.
7. Fraenkel, Much, and Starke: Ztschr. f. Hyg., 1911, lxix, 342.
8. Bonhoff: Arch. f. Hyg., 1904, l, 222.
9. Seiffert: Ztschr. f. Hyg., 1909, lxiii, 273.
10. Brion and Kayser: München. med. Wchnschr., 1902, xlix, i, 611.
———: Arch. f. klin. Med., 1905, lxxxv, 525.
11. Glaser: Ztschr. f. Hyg., 1910, lxvii, 459.
12. Hoffmann: Inaug. Dissert. Heidelberg, 1912.
13. Kraus and Stenizer: Wien. klin. Wchnschr., 1907, xx, 344; 1908, xxi, 645.
14. Johnston: Am. J. M. Sc., 1902, cxxiv, 187
15. Pratt: Boston M. and S. J., 1903, cxlviii, 151.
16. Cushing: Johns Hopkins Hosp. Bull., 1900, xi, 156.

## Bacillus paratyphosus A

The organisms known as *Bacillus paratyphosus* A were first clearly differentiated from Paratyphoid B by Brion and Kayser,[1] but it is probable that the first member of this group was described by Gwyn[2] as *Bacillus paracoli*.

The organisms differ culturally and serologically from Paratyphoid B and are isolated much more rarely. Morphologically they are small, rather thin bacilli, measuring 0.3 to 0.4 $\mu$ in width by $1\frac{1}{2}$ to 2 $\mu$ in length, with some longer forms measuring $2\frac{1}{2}$ to 3 $\mu$. Slightly enlarged on dextrose agar. *Agar colonies* small and delicate, in twenty-four hours $1\frac{1}{2}$ to 2 mm.

Fig. 80.—*Bacillus paratyphosus* A from twenty-hour culture on plain agar, stained with gentian-violet.

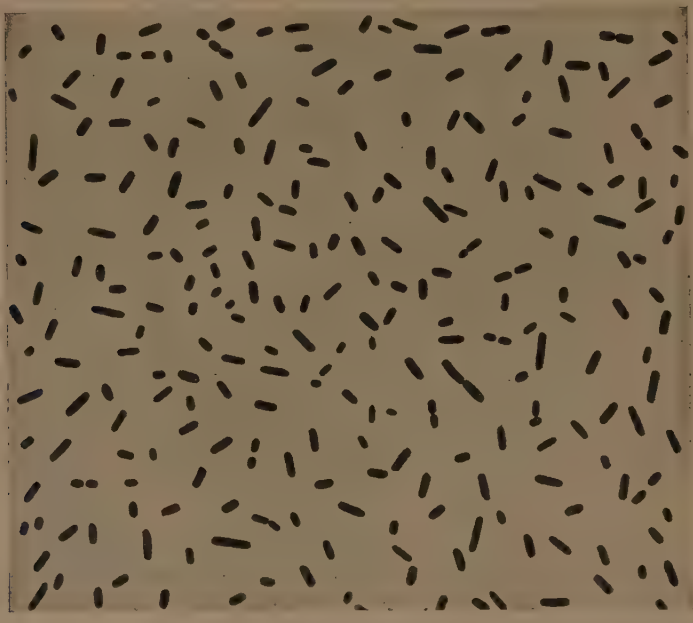

Fig. 81.—*Bacillus paratyphosus* A from twenty-hour culture on dextrose agar, stained with gentian-violet.

later considerably larger, 5 to 6 mm. in diameter when isolated. Growth on *agar slant* fairly abundant, smooth, and glistening. Development fairly heavy in *agar stabs* and on the surface. *Dextrose litmus agar colonies* flat and delicate, 2 to 3 mm. in size, with acid and gas bub-

bles in the agar. Acidity permanent. Moderate expansion on *dextrose litmus agar slants*. Delicate yellowish growth on *alkaline potato*. Cream-colored growth on *blood-serum*. *Gelatin colonies* small, ½ to 1 mm. in diameter in twenty-four hours. Under low power round and regular or irregular and feathery. Slight growth in *gelatin stabs*. No liquefaction of *gelatin*. Turbidity and scant sediment in *broth* and *peptone*. *Litmus milk* slightly acid in twenty-four hours, the acidity increasing in two to three days, but never being sufficient to coagulate the proteins. Acidity permanent, no return to alkali as with Paratyphoid B. The typical reaction resembles the litmus milk reaction with the typhoid bacillus. Acid produced also in plain milk.

*Fermentation Reactions.*—Gas and acid in dextrose. Gas formula $\frac{H}{CO_2} = \frac{3-4}{1}$. No fermentation of saccharose and lactose. Besides dextrose Paratyphoid A ferments galactose, levulose, mannose, rhamnose, maltose, arabinose, dulcite and mannite, but not xylose, melitiose, raffinose, and melibiose. Its attack on the carbohydrates is thus more limited than that of Paratyphoid B. No indol. Nitrites produced from nitrates, but not so much as with Paratyphoid B.

**Pathogenic** to mice, guinea-pigs, and rabbits, but requiring fairly large doses. By subcutaneous injection ⅓ c.c. of a twenty-four-hour broth culture kills mice in twenty-four hours. Intraperitoneal inoculation is fatal to young guinea-pigs in a shorter time. With rabbits subcutaneous injection produces necrosis and abscess formation. According to Rolly hemorrhagic enteritis can be produced in mice and guinea-pigs by feeding. Non-virulent for pigeons (Seiffert[3]). No definite evidence of a soluble toxin.

The type of disease produced by *Bacillus paratyphosus* A is like typhoid fever. The organisms are present in the stools and occasionally in the blood. Specific agglutinins are present in the blood-stream. The organism is not found in animals as *Bacillus paratyphosus* B is. It is found but rarely in the environment. It has been isolated, however, from water by Paladino Blondini[4] and May.[5] It has been obtained from sausage once by Schöne,[6] and from the organs of young pigs once by Uhlenhuth and Hübener.[7]

There is considerable confusion in regard to the differentiation of *Bacillus paratyphosus* A organism from Paratyphoid B. The original Gwyn culture produced a permanent acidity in litmus milk when first isolated. After several years' cultivation in the laboratory the milk reaction became identical with that of Paratyphoid B, an acidity reverting to alkali in a few days. Many other cultures of Paratyphoid A described as giving a permanent acidity in milk were also found to revert to alkali. The failure to ferment xylose may prove to be the only cultural distinction of value (Krumwiede). The groups can be separated by immunological reactions.

1. Brion and Kayser: München. med. Wchnschr., **1902, xlix, 1, 611.**
———: Arch. f. klin. Med., 1905, lxxxv, 525.
2. Gwyn: Johns Hopkins Hosp. Bull., 1898, ix, **54.**
3. Seiffert: Ztschr. f. Hyg., 1909, lxiii, 273.
4. Paladino Blondini: Ann. d'ig. sper., 1895, **xv.**
5. May: J. Trop. M. and Hyg., 1911, xv, **1.**
———: Jour. Roy. Inst., 1904, xvii.
6. Schöne: Berl. klin. Wchnschr., 1909, lxvii, **1.**
———: Ztschr. f. Hyg., 1910, lxv, 1.
7. Uhlenhuth and Hübener: *In:* Handb. d. Path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, iii, 1005.

### Bacillus paratyphosus C

Isolated by Uhlenhuth and Hübener in young pigs sick of swine-plague. Has all the reactions of the intermediate group. Serologically not identical with either *Paratyphosus B*, or Gärtner, but a distinct organism. Found in the feces of man by Hübener and Viereck.

Uhlenhuth, Hübener, Xylander, and Bohtz: Arb. a. d. K. Gsndhtsamte., 1909, xxx, 217.

### Indolformers

Paratyphoid B strains producing indol have been found by a number of observers including Andrejew[1] in cultures from sheep intestines, Huber from horse intestines, Huber and Horn[2] from cattle feces, Schmidt from intestines of swine, Sobernheim and Seligmann[3] from various kinds of meats. No grouping has thus far been devised for them. Indolformers, called *Bacillus paratyphosus* N, have been reported by Klüchin.[4]

1. Andrejew: Arb. a. d. K. Gsndhtsamte., 1910, xxxiii, 363.
2. Huber and Horn: Centralbl. f. Bakteriol. [etc.], 1911, lxi, 452. Ztschr. f. Infektionskr. Haustiere, 1911, x.
3. Sobernheim and Seligmann: Ztschr. f. Immunitätsforsch., 1910, vi, 401; 1910, vii, 342. Sobernheim: Deutsche med. Wchnschr., 1910, xxxvi, 1552.
4. Klüchin: Centralbl. f. Bakteriol. [etc.], 1924, xciii, 495.

**Paratyphoids in Pseudotuberculosis.**—*Bacillus paratyphosus* B produces pseudotuberculosis in guinea-pigs. M. Neisser has observed an epidemic, studied by Eckersdorff.[1] Dieterlen[2] has shown that *B. paratyphosus* B produces pseudotubercles in the spleen of guinea-pigs following subcutaneous infection or feeding. Simon has also reported that inoculation with *paratyphosus* B produces necrotic areas in spleen and liver of guinea-pigs.

1. Eckersdorff: Arb. a. d. K. Inst. f. exper. Therap. zu Frankf. a. M., 1908, iv, 61.
2. Dieterlen: Arb. a. d. K. Gsndhtsamte., 1909, xxx, 429.

### BACILLUS PSITTAKOSIS Nocard

The psittacosis bacillus was discovered by Nocard[1] in an epidemic of enteritis, 49 cases with 16 deaths, and traced to infection from the dried bone-marrow of a parrot which had died of epidemic enteritis. The organisms were cultivated from the bone-marrow and Nocard produced the disease in parrots by feeding. He gave the name "psittokosis" to the disease. Nocard's bacillus was subsequently found in the heart blood of a patient dead of psittacosis.

Doubt has been raised as to the etiological rôle of Nocard's bacillus, both in parrots and man, by Boehm, Selter, and Finkler.[2] According to Uhlenhuth, Haendel, and Schern,[3] there can be no question, but that Nocard's bacillus is the cause of psittacosis in parrots, but some doubt may be reasonably expressed as to its occurrence in man.

The organisms occur in the heart blood of parrots, in the intestinal tract, bone-marrow, and liver. They have close immunological relations with the Aertrycke bacillus as shown by Durham[4] and De Nobelle,[5] being reciprocally agglutinated by sera.

Morphology, cultured reactions, and biological requirements like Paratyphoid B. Agglutinins are easily produced in animals, but results are not always the same as far as grouping is concerned.

The organism is virulent to ordinary laboratory animals by subcutaneous and intraperitoneal injection, also per os. It is virulent to parrots, killing them in one to five days with the organism in the internal organs. Heuser[6] states that the filtrates of old cultures have the same virulence as the cultures themselves.

1. Nocard: Publ. de cons. d'hyg., 1893, March 24.
2. Boehm, Selter, and Finkler: Klin. Jahrb., 1910, xxiii.
3. Uhlenhuth, Haendel, and Schern: Deutsche tierärztl. Wchnschr., 1909, No. 24.
4. Durham: Brit. M. J., 1898, ii, 1797.
5. De Nobelle: Ann. Soc. méd. de Gand., 1899 and 1902.
6. Heuser: Ztschr. f. Hyg., 1910, lxv, 9.

### BACILLUS PESTIS-CAVIÆ Wherry

Reported originally by Smith and Stewart[1] from guinea-pigs with pseudotuberculosis and extensively investigated by Wherry,[2] who found it to be identical with a rat virus called "azoa." Known also as *Bacillus choleræ-caviæ*.[3] A similar organism was isolated by Carter in Baltimore from infectious endometritis in guinea-pigs. Description from Wherry.

**Morphology.**—Actively motile rod-shaped organisms with rounded ends, showing bipolar staining with carbol thionin. Most frequently 0.8 $\mu$ by 1.8 $\mu$ in size, although smaller coccoid forms, 0.8 by 0.8 $\mu$ and longer forms, 0.8 by 2.8 $\mu$ may be seen. Gram-negative.

Grows readily at 25° to 28° C. and at 37° C.

**Cultural Characters.**—*Agar* or *Litmus Lactose Agar Plates.*—Colonies circular, white, slightly elevated, punctate, up to 0.5 to 1 mm., appearing in twenty-four hours at 37° C. When well separated they may spread out to 3 to 4 mm. in diameter. They appear light brownish by transmitted light and produce no change in lactose.

*Agar Slants.*—Similar colonies when well separated. If very numerous and closely aggregated they are minute and transparent. The growth is not viscous.

The growth from animal tissues appears frequently in the form of "ring-colonies," the bacteria being piled up in a white ring about a clear central space.

Cloudiness in *sugar-free broth* and a faint but distinct indol reaction after ten days' growth at 37° C. In old broth cultures coarse stalactites may be produced.

Good growth in +1.5 *gelatin* at 25° to 28° C. without liquefaction. No visible change in *milk* at first, after seven to ten days at 37° C. the culture appears opalescent, later it is turned to a clear, yellowish fluid.

*Carbohydrates.*—Acid and gas production found by Wherry in dextrose, levulose, galactose, maltose, and mannite. None from saccharose, lactose, or inulin. Gas formula $\frac{H}{CO_2} = \frac{2}{1}$.

**Pathogenic** to guinea-pigs, rats (*Mus norvegicus*), mice (*Mus musculus*), and rabbits. In guinea-pigs of 300 to 500 gram weight subcutaneous inoculation of 2 c.c. of a twenty-four-hour sugar-free broth culture causes death in three to six days. At autopsy there is an extensive exudate at the site of inoculation, hemorrhagic-fibrinous and purulent in character, with a serogelatinous exudate in the groins. The spleen is enlarged and soft when the animal lives five to six days. Hydrothorax and fibrinopurulent pericarditis may be found. The internal organs are generally congested. Smaller doses, 0.25 to 0.75 c.c., usually produce a localized abscess followed by necrosis, sloughing, and recovery. Intraperitoneal injection produces death more rapidly with marked fibrinopurulent peritonitis and septicemia. In rats, 4 c.c. of twenty-four-hour sugar-free broth cultures must be injected subcutaneously into adult rats (150 to 350 gms.) in order to produce any uniform lethal effects. When they succumb death occurs after eight to twelve days. There is abscess formation at the site of injection, the spleen is enlarged, sometimes four to five times its normal size, soft, and usually shows numerous yellowish-white specks of necrosis. If the rat survives and is chloroformed, minute yellowish-white nodules, 0.5 to 2 mm. in size may be found in the spleen. The liver may be congested or show numerous yellowish-white foci of necrosis. Occasionally there are dark coffee-colored areas of hemorrhage in the lungs and sometimes hydrothorax. Rats and mice are uniformly susceptible to infection by feeding. They either succumb rapidly to an acute septicemia without any marked anatomical changes, or when more resistant show the anatomical changes in the spleen and liver, and sometimes hemorrhage in the lungs, which are seen following subcutaneous inoculation.

In rabbits localized abscesses are produced by subcutaneous inoculation.

**Remarks.**—This species was regarded by Wherry as closely related to *Bacillus enteritidis* of Gärtner. The original host of the organism seems to be the guinea-pig (*Cavia aperea*).

1. Smith and Stewart: J. Bost. Soc. M. Sc., 1897, i, 12.
2. Wherry: J. Infect. Dis., 1908, v, 519.
3. Wherry: Pub. Health Rep. U. S. Mar. Hosp. Serv., 1908.

### Bacillus of calf diarrhea

Several infections occur in newborn calves, a diarrhea known in this country and England as "scours," a septicemia without intestinal involvement, and an inflammation of the lungs known as septic pneumonia. From the intestinal discharges in these cases a great many different organisms have been isolated, such as Jensen's *Paracoli bacilli* and Poel's *Pseudocoli bacilli*. Organisms belonging to the intermediate group have been obtained from some of these epidemics, as by Thomassen[1] in 1897 in Holland. He produced a septicemia in animals by subcutaneous injection and by feeding, this septicemia resembling the natural disease. A disease similar to Thomassen's in Holland was later described in America by Mohler and Buckley, who obtained organisms also of the paratyphoid group.

Organisms of this type have also been isolated by Joest[2] and by Uhlenhuth and Hübener. They represent a more or less definite member of the paratyphoid group and are, in many instances, agglutinated by Gärtner, or Aertrycke serum. In morphology, cultural, and biological reactions, they are identical with paratyphoid B or *Bacillus enteritidis* of Gärtner. They live in gelatin at room temperature up to thirteen months, on agar and in broth at ice-box temperature up to twenty-six months. They are destroyed by heating to 65° C. one hour. They have the same pathogenicity for animals that paratyphosus B and Gärtner bacilli have, killing laboratory animals by subcutaneous, intravenous, and intraperitoneal inoculation, and alimentary administration. Pigeons develop abscesses after intramuscular inoculation. Feeding gives rise to hemorrhagic enteritis. Subcutaneous and intravenous inoculation to septicemia.

Serological reactions have not resulted in establishing a definite group of organisms of this character in calves.

1. Thomassen: Ann. de l'Inst. Pasteur, 1897, xi, 523.
2. Joest: Schweineseuche and Schweinepest. Jena, 1906. Centralbl. f. Fleisch-u. Milchhyg., 1905, No. 10.

### Danysz's Bacillus of Rat Plague

Cultivated by Danysz[1] in 1900 from an epidemic among field and woods mice. Intensely virulent for rats and mice and often used as a virus for exterminating them. Similar organisms isolated by Issatchenko,[2] Bahr,[3] and Dunbar and Trautmann.[4] According to Trautmann, Danysz's bacillus and Dunbar's bacillus are identical with the Gärtner bacillus. Subsequently Issatchenko's bacillus was recognized as belonging to the Gärtner group. Bahr's bacillus is apparently a member of this group, as well as the organism present in rat virus used in England, the Liverpool virus.

**Pathogenic Action.**—The rat bacilli of this group are marked by high pathogenicity for wild and tame rats, both by inoculation and by feeding. They are also virulent for white and gray mice, guinea-pigs, and rabbits. In rats feeding leads to the death of the animals in five to eight days; subcutaneous and intraperitoneal inoculation somewhat earlier. The pathological changes are marked and somewhat resemble the lesions produced by *Bacillus pestis*. The lymph-glands are everywhere swollen; there is an excess of turbid serous fluid in the peritoneal cavity, the spleen is enlarged, the surface of the abdominal organs covered with fibrinopurulent material. Both liver and spleen may show miliary and submiliary necroses. Trautmann[5] reports that many rats are insusceptible to rat virus. This may be due to an acquired immunity. The virus loses its strength rapidly, this often being restored by animal passage. The organisms are also virulent for German marmots, hens, and calves.

The rat bacilli rarely infect man. Gaffky[6] has reported the illness of one individual who handled the virus. Handson, Williams, and Klein have observed a number of cases of gastro-enteritis which they traced to contamination of food with Liverpool virus. In several instances the organism was obtained from the dejecta.

1. Danysz: Ann. de l'Inst. Pasteur, 1900, xiv, 193.
2. Issatchenko: Centralbl. f. Bakteriol. [etc.], 1898, xxiii, Orig., 873. *Ibid.*, 1902, xxxi, 26.
3. Bahr: Centralbl. f. Bakteriol. [etc.], 1905, xxxix, 263. *Ibid.*, 1909, lii, 44. *Ibid.*, 1910, liv, 228.
4. Trautmann: Ztschr. f. Hyg., 1906, liv, 104.
5. Trautmann: Ztschr. f. Hyg., 1903, xlv, 139.
6. Gaffky: Klin. Jahrb., 1909, xx, 55. Festschrift. 60. Geburtst. v. Robert Koch, Jena, 1903.

### Bacillus abortus equi Meyer and Boerner

Bacteria belonging to the hog-cholera or paratyphoid group were first isolated from cases of abortion in mares by Kilborne and Smith,[1] Lignières,[2] Lignières and Zabala, Good,[3] Van Neelsbergen,[4] De Jong[5] and a number of other observers. The causative organism was isolated by Meyer and Boerner[6] from the aborted placentas and from the vaginal mucosa of a number of aborting mares. They give the following description:

**Morphology.**—Short, ovoid, rather thick rod, with rounded ends, and with a tendency to bipolar staining when examined from tissues. Thickness 0.2 to 0.5 $\mu$, length 0.5 to 1.5 $\mu$.

No bipolar staining in cultures, but frequently long filament-like involution forms seen.

No spores.

**Motility** active. Flagella numerous, seen by dark-field illumination.

**Staining Reactions.**—Stains well with ordinary anilin dyes. Gram-negative.

Not acid-fast.

Cultivated easily on the usual media.

**Cultural Reactions.**—*Agar Plates.*—Colonies small, round, slightly raised in twenty-four hours at 37° C. After forty-eight hours superficial colonies round, surface concentrically ringed, center slightly raised, margins irregular, flattish grayish-white disks, finely granular. Deep colonies lenticular, opaque, with knobbed periphery.

*Agar Slant.*—Grayish-white, glistening, somewhat wrinkled growth in twenty-four hours. Growth slightly membranous in forty-eight hours.

*Agar Stab.*—Nail-head growth, surface raised with lobulated edges. Beaded growth along line of puncture.

*Glycerin* or *Glucose Agar.*—Growth the same as on plain agar, except more rapid, abundant, shining, and moist.

*Gelatin Plates.*—Colonies very small and just visible in forty-eight hours, later the size of a pin-head, reaching maximum size in about a week. Superficial colonies round or slightly lobulated, transparent, flat, center dark, and surrounded by fine granular concentric rings. Surface somewhat wrinkled and finely granular. Deep colonies lenticular.

*Gelatin Stab.*—Nail-head growth. Surface growth rapid and abundant, line of puncture beaded. No liquefaction.

*Loeffler's Blood-serum.*—Membranous, somewhat adherent growth.

*Potato.*—No growth on acid potato. Moist, abundant, light straw-colored growth on alkaline potato or on potato with 5 per cent. glycerin added.

*Broth.*—Uniform turbidity in twenty-four hours, a thin veil in three to four days on the surface, later thickening into a pellicle. Viscid sediment. Optimum reaction between +1 per cent. and +1.5 per cent. to phenolphthalein.

*Glycerin Broth* (4 to 5 per cent.).—Growth more rapid than in plain broth, pellicle formed sooner, sediment more abundant.

*Glucose Broth* (1 per cent.).—Growth like that in glycerin broth. Acid and gas produced in small quantities.

*Dunham's Peptone.*—Growth slower than in plain broth, pellicle formation poor and often entirely absent.

*Milk.*—No change in first ten days. Then a gradual clearing, the whey yellowish and clear, the casein precipitated.

*Litmus Milk.*—Blue color slightly diminished in twelve hours, somewhat reddish, later more intense.

*Litmus Whey.*—After twelve hours reddish, changed in forty-eight hours deep blue on fifth day.

*Endo's Fuchsin Agar.*—Small white colonies in twenty-four hours, 1 to 2 mm. in size, later typical whitish disks.

No indol. No $H_2S$.

**Fermentation Reactions.**—Acid and gas in glucose, maltose, dulcite, galactose, arabinose, mannose, levulose (fructose), sorbite, rhamnose, xylose, and mannite.

No reaction with lactose, saccharose, adonite, raffinose, glycogen, dextrin, inulin, erythrite, and sorbose. Slight acid with glycerin. Gas in glucose H 67 per cent., $CO_2$ 33 per cent., in dulcite H 75 per cent., $CO_2$ 25 per cent., in mannite H 78 per cent., $CO_2$ 22 per cent.

**Thermal Death-point.**—Killed at 60° in eight minutes.

Destroyed by 1 per cent. carbolic solution in seven minutes, in 1 per cent. lysol in four minutes.

**Pathogenic Action.**—Pure cultures produce abortion in pregnant cows and goats by intravenous injection; intravenous and intravaginal injection produce abortion in pregnant sows. The organism can be obtained from the aborted fetuses and from the uterus of the mother. Agglutinins and complement-fixing substances can be demonstrated in the blood. Organisms virulent in small doses for guinea-pigs and rabbits by subcutaneous, intraperitoneal, intravaginal, and stomachial administration, the animals aborting when pregnant. Virulent also for white mice, white rats, and pigeons, but not for chickens.

Culture filtrates not poisonous to rabbits, guinea-pigs, or mice. Filtrates moderately hemolytic for blood of sheep, rabbit, and guinea-pig.

This organism can be differentiated by agglutination and complement-fixation from *Bacillus paratyphosus* A and B and from *Bacillus enteritidis* of Gärtner.

1. Kilborne and Smith: Dep. Agr. Bur. An. Ind., 1893, Bull. No. 3, p. 49 and 53.
2. Lignières: Rec. de méd. vét., 1905, lxxxii.
3. Good: Kentucky Agr. Exper. Sta., 1912, Bull, 165.
4. Van Neelsbergen: Tijdsch v. Veeartsenijk, 1912.
5. De Jong: Centralbl. f. Bakteriol. [etc.], 1912, lxvii, 148.
6. Meyer and Boerner: J. Med. Research, 1913–14, xxix, (n. s. xxiv), 325.

### BACTERIUM ANATUM Rettger and Scoville

Isolated by Rettger and Scoville from the heart, liver, and lungs of ducklings suffering from a disease known as "Keel" and resembling bacillary "white diarrhea" of chicks, and from various organs in adult ducks.

**Morphology.**—Short rod-shaped organism resembling the shorter varieties of *Bacillus coli* and *Bacillus enteritidis* rather than *Bacillus typhosus*. Average size 0.5 by 1 to 2 $\mu$.

**Motility** active from several peritrichic flagella, the exact number not being determined.

**Stains** readily by usual dyes. Gram-negative.

Fig. 82.—*Bacterium anatum* from plain agar twenty-four hours old, stained with gentian-violet.

Fig. 83.—*Bacterium anatum* from dextrose agar twenty-four hours old, stained with gentian-violet.

**Cultivated** easily on ordinary media as a facultative anaërobe, having a very decided preference for atmospheric oxygen.

*Gelatin and Agar Plates.*—Colonies like those of other members of this group. Growth is

slightly heavier than that of *Bacillus typhosus* and less luxuriant than the colonies of *Bacillus coli*.

*Slant Agar*.—Growth moderately abundant in twenty-four hours, indistinguishable from that of *Bacillus paratyphosus* B and *Bacillus enteritidis*. The growth becomes quite luxuriant and opaque after the first two days, and often shows more or less wrinkling eventually near the base. When inoculation is made over the entire surface with blood from the infected organs, or with a light suspension of the organisms in water or other liquid media, discrete colonies are formed over the agar surface which in the early stages very closely resemble those of *Bacillus pullorum* and which remain separate even after twenty-four to thirty hours at incubator temperature. The older colonies are decidedly larger and more opaque than those of *Bacillus pullorum*.

*Gelatin Stab*.—Light growth along needle-track, with little or no spreading on the surface. No liquefaction.

*Broth*.—Heavy clouding and surface pellicle in twenty-four to thirty-six hours at 35° to 37° C. No appreciable odor.

*Litmus Milk*.—Faintly acid within forty-eight hours, but gradually changing to alkaline. Marked alkalinity at the end of twelve to fourteen days. Reaction like that of *Bacillus sanguinareum* (Moore) and *Bacillus paratyphosus* B.

*Potato*.—Growth varies from scanty with no discoloration of the medium to heavy and moist. In two to three days the latter becomes light brown with a discoloration of the potato.

No indol in Dunham's peptone or in tryptophan broth.

No Voges-Proskauer reaction.

Nitrates reduced to nitrites in twenty-four hours at 37° C.

All strains isolated by Rettger and Scoville were able to utilize the nitrogen of ammonia, nitrate, urea, creatin, and nucleic acid, but failed to attack urea.

Gas production determined in Dunham fermentation tubes, with brom cresol purple for the detection of acidity at 37° C. Acid production with glucose, levulose, maltose, galactose, dextrin, arabinose, rhamnose, inosit, xylose, dulcitol, mannitol, and glycerol.

Gas production with percentage of gas produced measured by the Frost gasometer in glucose 40, levulose 25, maltose 10, galactose 35, dextrin 5 to 10, arabinose 10 to 15, rhamnose 10 to 15, inosit 10, xylose 10, mannitol 20, dulcitol 35 to 40.

One strain failed to produce acid or gas with xylose and one strain failed to produce gas, but did produce acid.

Hydrogen-ion concentration in peptone, pH =4.8–4.9, in synthetic phthalate medium of Clark and Lubs, pH =4.6.

Agglutinins produced in rabbits by subcutaneous injection of killed cultures followed by a suspension of living organisms. The serum of rabbits immunized with *Bacillus anatum* agglutinates *Bacillus paratyphosus* B. *Bacillus anatum* also agglutinated by the serum of animals immunized by *Bacillus paratyphosus* B.

**Pathogenic** to chicks by subcutaneous inoculation. At autopsy there is a swollen reddened area with a small amount of fibrinous exudate at the site of inoculation and the organism can be recovered in large numbers from the heart's blood and liver. Subcutaneous injection in young rabbits and half-grown guinea-pigs produces a tumor-like mass which breaks down and yields a fluid resembling ordinary pus. Virulent to young ducklings a few days old by subcutaneous injection. At autopsy there is a nodula swelling at the site of inoculation with a fibrinous exudate. The internal organs appear normal. *Bacillus anatum* can be recovered from the heart's blood and spleen in large numbers.

Rettger and Scovile: J. Infect. Dis., 1920, xxvi, 217.

### MORGAN'S BACILLUS No. 1

Described by Morgan[1] from cases of dysentery.

A motile, multiflagellated Gram-negative bacillus, slightly smaller than the typhoid bacillus which does not liquefy gelatin and renders milk alkaline in about fifteen days. Litmus whey is also rendered alkaline. Acid and gas produced in the monosaccharids, glucose, levulose, and galactose. No acid or gas in mannite, dulcite, maltose, dextrin, cane-sugar, lactose, inulin, amygdalin, salicin, arabinose, raffinose, sorbite, or erythrite. Indol produced in considerable quantity.

*Virulent* to young rats and young rabbits by feeding agar cultures. The animals develop a violent diarrhea and die in twenty-four hours. The bacilli are found in the spleen and heart's blood. Non-virulent by intravenous and intraperitoneal inoculation, although filtrates from broth cultures sometimes cause diarrhea and death in rabbits in twelve hours when injected intravenously.

Found in cases of dysentery, but not agglutinated by the serum of the patient. Not definitely shown to be the cause of dysentery.

Levine,[2] who has made a careful study of cultures from the stools of infant diarrhea cases, reports that there are a number of organisms in the dejecta closely related to the Morgan bacillus. Of these, he makes three groups distinguished by cultural and serological tests.

1. Morgan: Brit. M. J., 1906, i, 908. 1907, ii, 16.
2. Levine: Am. J. Pub. Health, 1925, xxv, 17.

### Classification of Bacterium Group II

In the lack of agreement as to serological reactions the only method of classification which can be suggested is according to source:

*Paratyphoid Group*

Primary in Man

*Paratyphosus* A.
*Paratyphosus* B.
Morgan's Bacillus No. 1.

Primary in Cattle

*Bacillus enteritidis.*
Bacillus of calf diarrhea.
*Bacillus nodulificans.*

Primary in Veal

*Bacillus Aertrycke.*

Primary in Swine

*Bacillus suipestifer.*
Pig paratyphoids.

Primary in Rats

Danysz rat bacillus.

Primary in Mice

*Bacillus typhi murium.*

Primary in Parrots

*Bacillus psittakosis*

Primary in Horses

Bacillus of equine abortion.

Primary in Fowls

*Bacillus anatum.*

Primary in Guinea-pigs

*Bacillus pestis caviæ.*

### Bacterium Group III

Non-liquefying, producing acid, no gas with carbohydrates. The typhoid-dysentery group:

*Bacillus typhosus* Gaffky.
" *dysenteriæ* Shiga.
" " Flexner.
" " Strong and Musgrave.
" " Hiss and Russell.
" " Schmitz.
" *alkalescens* Andrews.
" *dispar* Andrews.
" *ambiguus* Andrews.
*Paradysentery bacilli* A and B of Mita.
*Bacillus acidoformans* Sternberg.
*Bacterium anaërogenes* Lembke.

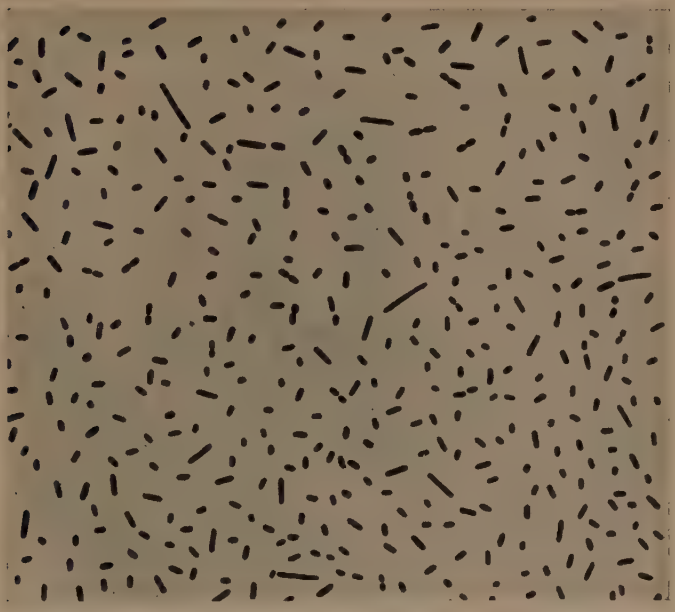

Fig. 84.—*Bacillus typhosus* from plain agar twenty-four hours old, stained with gentian-violet.

### BACTERIUM TYPHI-ABDOMINALIS Gaffky

The etiological agent in typhoid fever was first observed by Klebs,[1] Eberth,[2] and Koch[3] in the organs of individuals dead of the disease, such as the spleen, mesenteric glands, liver, and kidney. First obtained in gelatin cultures by Klebs (see Gay) and on other solid media by Gaffky.[4] Since then found in all characteristic cases of the disease in the stools and blood during life and in the various organs after death. Usually called *Bacillus typhosus*.

**Morphology.**—Long, rather slender bacilli, considerably more delicate than *Bacillus coli*. On plain agar twenty-four hours old the organisms usually measure 0.4 to 0.5 $\mu$ in breadth and 1 to 3 $\mu$ in length, but longer forms measuring 4 to 5 $\mu$ in length are not uncommon. Threads measuring 12 to 15 $\mu$ are frequently seen, especially on the surface of broth cultures and on gelatin and potato cultivated at low temperatures. In strains cultivated from the blood threads are common in the first generation (F. Müller[5]). In the body the bacilli may be thicker and plumper than on artificial cultures (Bail and Rubritius[6]), but this is not always so. In some strains *Bacillus typhosus* is differentiated morphologically from *Bacillus coli* with

great difficulty. On dextrose agar in twenty-four hours the organisms are considerably thicker and longer, measuring 0.7 by 3 to 4 $\mu$ in size. Short coccoid forms and long forms measuring 0.6 to 0.7 by 5 to 7 $\mu$ are also common on dextrose agar.

**Motility** characteristic. The organisms have a progressive undulatory or rotating movement and change their location with great rapidity. The long threads have a snake-like motion. Very rarely true strains of *Bacillus typhosus* from the human body have been reported without motility (Kutscher[7]). Motility is best observed at 22° C. in hanging drops from young cultures in slightly alkaline broth. Different observers prefer different media from which to observe motility. Lösener,[8] used coagulated serum slants; Terni,[9] 3 per cent. glycerin broth free from peptone of an acidity of about 0.1 per cent. HCl; Germano and Maurea.[10] 2 per cent. glucose broth. Motility is frequently lost in alkaline media.

**Staining Reactions.**—*Bacillus typhosus* stains easily by the ordinary anilin dyes, methylene-blue, gentian-violet or dilute carbolfuchsin. Gram-negative. The flagella are best stained by the methods of Zettnow and Peppler although satisfactory preparations may also be obtained by the older methods of van Ermengem, Loeffler, etc. The flagella number 10 to 12 and have a peripheral arrangement about the body of the organism.

**Cultivation.**—The typhoid bacillus may be cultivated without difficulty from the organs of individuals dead of the disease or from the complicating lesions. It grows readily on the

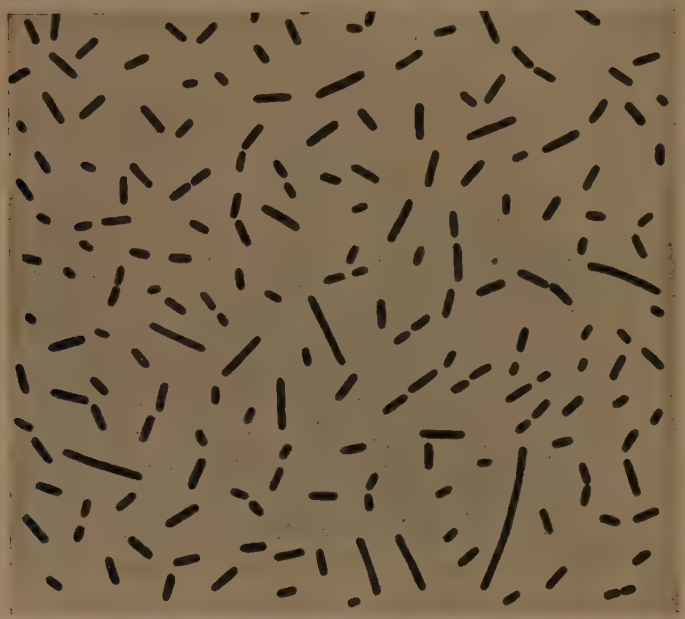

Fig. 85.—*Bacillus typhosus* from dextrose agar twenty-four hours old, stained with gentian-violet.

usual artificial media. It is easily cultivated from the blood. In the stools it is associated with the intestinal bacteria and does not appear on culture media of the usual composition, special media being necessary for its isolation. It grows best at 37° C., slowly at 20° C., poorly at 9° to 15° C. It grows both aërobic and anaërobic conditions, on neutral, weakly acid or weakly alkaline media.

**Cultural Characters.**—*Plain Agar Plates.*—Colonies appear on the surface usually in twenty-four hours at 37° C.; small, round, regular, rather thin, whitish or bluish white in color, $\frac{1}{2}$ to 2 mm. in diameter. Under low power they are translucent with dense centers and thin margins, edges entire. Deep colonies small almost pin-point. Under low power usually oval or disk-shaped. The surface colonies increase somewhat in size, but seldom more than 3 to 4 mm. in the first few days. In old plates isolated colonies may become quite large, 0.5 to 1 cm. in diameter, with crenated edges. Deep colonies also increase in size. In general the colonies are smaller, flatter and less opaque than colonies of *Bacillus coli.*

*Plain Agar Slant.*—Rather delicate, flat, smooth, glistening, translucent growth in twenty-four hours. The growth increases in extent in two to three days, spreading over the surface of

the agar, the edges being frequently jagged and crenated. Later the growth is slightly thicker, but it remains somewhat translucent even in old cultures.

*Plain Agar Stab.*—Delicate growth along the line of inoculation and on the surface in twenty-four hours. The growth increases moderately, but remains somewhat delicate even in old cultures.

*Dextrose Litmus Agar Plates.*—Surface colonies appear in twenty-four hours, rather thin and delicate, measuring about 1 mm. in diameter. Under low power the colonies are slightly granular with dark centers, thin margins, edges entire. Deep colonies barely visible. Under low power granular, either round or biconvex disks. Agar highly acid. Older colonies are somewhat larger in size, but seldom measure more then 2 to 4 mm. even in old plates. Agar usually remains acid in reaction.

*Dextrose Litmus Agar Slant.*—Rather delicate, smooth, glistening growth in twenty-four hours with a moderately acid reaction. In two or three days the growth is increased in extent but it always remains thin and delicate even in old cultures. Agar usually remains acid in reaction.

*Blood-serum.*—Delicate, smooth, glistening growth in twenty-four hours. The growth increases slowly, never becomes very abundant. In old cultures it may be somewhat raised and whitish.

*Potato.*—The character of the growth depends upon the composition of the potato. In some instances no visible growth is evident, microscopic examination revealing a multiplication of the organism. In other cases a delicate, dry, whitish growth appears. Very rarely the growth may be yellowish or pale brown especially in old cultures on alkaline potato.

*Gelatin Plates.*—Colonies barely visible in forty-eight hours as minute points; under low power round, regular, and uniform with sharply defined edges. The colonies grow slowly and at the end of five to six days may be only 1 mm. in diameter. Older colonies under low power may have dark centers and irregular margins with crenated edges. Sometimes the colonies may be moruloid with leaf-like margins. Occasionally a mass of daughter colonies develops about the original colony. No liquefaction.

*Gelatin Stab.*—Delicate line growth and faint surface growth in twenty-four hours. The growth always remains delicate even after some days. No liquefaction.

*Broth.*—Turbidity and sediment in twenty-four hours. No scum. Turbidity and sediment increase markedly, but the growth is never so abundant as with *Bacillus coli.*

*Peptone.*—Delicate growth, turbidity and sediment, in twenty-four hours. Some increase after a week or ten days. Growth never heavy.

*Litmus Milk.*—Growth rapid. Acid reaction faint in twenty-four hours, somewhat more marked in forty-eight hours. The acidity never becomes enough to coagulate the milk. In some strains of genuine typhoid bacilli the reaction becomes alkaline after some days. The length of time required for the alkaline reaction to develop varies from two to three weeks.

*Litmus milk-whey* remains clear and is very slightly reddened (Petruschky[11]).

*Dextrose Fermentation Tube.*—Turbidity and sediment in bowl in twenty-four hours, growth extending up into the closed arm on the second or third day. No scum. Reaction acid at the end of three days. In saccharose and lactose the turbidity and sediment are the same, but the arm remains clear and the reaction neutral or alkaline.

Other carbohydrates fermented to an acid reaction besides dextrose are galactose, levulose, mannite, maltose, and sorbite. Dextrin is not usually attacked, but Winslow, Kligler, and Rothberg[12] report acidity from it. Xylose is fermented, the time of acid production varying. Some strains produce acid in the first twenty-four hours, others after five to thirteen days (Krumwiede, Kohn, and Valentine[13]).

No acid production with saccharose, lactose, inulin, and glycerin.

**Special Media.**—A large number of special media have been devised to enable the observer to differentiate accurately between *Bacillus typhosus* and the members of the intestinal group of bacteria. Some of these contain substances which have an inhibitory action on the other closely similar bacteria, and these are especially valuable in the isolation of the typhoid bacillus from the stools. The most important of these are the following:

*Wurtz's Lactose Litmus Agar.*—The typhoid bacillus produces good-sized colonies, but having no action on lactose, produces no acid, the surrounding medium remaining blue. Organisms fermenting lactose produce an acidity in the agar about the colonies and, in some instances, bubbles of gas.

*Conradi-Drigalski medium,* containing lactose, litmus, and 10 per cent. crystal violet. Colonies of *Bacillus typhosus* small, blue, and transparent and remain blue. Colonies of *Bacillus coli* produce an acidity in the medium with bubbles of gas, and are large, red, and opaque.

*Rothberger's Neutral-red Glucose Agar.*[14]—In shake or stab-cultures the typhoid bacillus grows, but produces no color change. *Bacillus coli* and other intestinal bacteria reduce the red, decolorize the medium, and produce bubbles of gas.

*MacConkey's Bile-salt Medium.*—*Bacillus typhosus* grows abundantly without precipitation of the bile-salts. *Bacillus coli* produces acid from the lactose, precipitates the bile-salts, and clouds the medium.

*Barsiekow's Lactose Nutrose Medium.*—*Bacillus typhosus* causes no change. *Bacillus coli* produces an acid reaction and coagulation.

*Russell's double sugar*, containing glucose and lactose with litmus or Andrade indicator. *Bacillus typhosus*, together with *Bacillus paratyphosus* A and B and dysentery bacilli, grow without color on the slants with a deep red color where partial anaërobic conditions exist and acid is produced. No gas bubbles are produced by *B. typhosus* or by any dysentery bacilli. *Bacillus coli* produces acid and gas bubbles throughout.

*Endo medium* containing lactose and saturated alcoholic fuchsin. Robinson and Rettger's modification and Kendall's modification. Colonies of *Bacillus typhosus* are colorless. Those of *Bacillus coli* become red.

*Krumwiede's Brilliant Green.*—Colonies of the typhoid bacillus are large, have a snow-flake appearance in oblique light with serrated edges. With artificial light under a hand-lens the colonies have a texture resembling a coarse woolen fabric. *Bacillus coli* and the dysentery bacilli are inhibited by certain dilutions of Krumwiede's medium.

**Resistance.**—Cultures of *Bacillus typhosus* are quite viable, remaining alive on culture-media for long periods. Destroyed by heat in a short time, at 56° C. in ten minutes (Sternberg). Killed easily by disinfectants, by 5 per cent. carbolic acid or 1 : 500 bichlorid of mercury in five minutes.

**Chemical Products.**—*Bacillus typhosus* does not ordinarily produce indol. Experiments by Peckham[15] indicate that after long-continued cultivation on protein-rich media a moderate indol reaction may be given. More recently Bull and Pritchett[16] have reported a strain agglutinating up to a dilution of 1 : 20,000 with typhoid serum in which positive indol reactions were present. It produces some phenol, hydrogen sulphid, and nitrites. In certain protein solutions (peptone broth) it produces protein-chrome from extensive splitting of the protein. After slight acidification with acetic acid the solution gives a red-violet color with chlorin or bromin. Erdmann and Winternitz[17] claim that this protein-chrom reaction is given also by the paratyphoid bacilli, not by *Bacillus coli.*

**Pathogenic Action.**—*Bacillus typhosus* is virulent for a number of small animals, such as mice, rats, rabbits, and guinea-pigs. The animals develop a septicemia from subcutaneous and intravenous inoculation and the organisms are distributed throughout the body. After intraperitoneal injection a bloody purulent exudate is found in the peritoneal cavity containing many typhoid bacilli which are present as well in the blood. The pathological picture is like that produced by *Bacillus coli.* Petruschky[18] says that the minimum lethal dose for mice by intraperitoneal inoculation lies between 0.15 and 0.3 mg., that is, 10 to 15 mg. per kilo body weight. With subcutaneous injection the dose is 5 to 6 times larger. In rats and rabbits the fatal dose is about the same, with guinea-pigs somewhat less, 5 to 10 mg. pro kilo. Subsequently, Pfeiffer and Kolle[19] have shown that in freshly isolated cultures the virulence for 300-gram guinea-pigs varies between $\frac{1}{8}$ and $\frac{1}{60}$ of an oesa of a twenty-four-hour agar culture, an oesa being equivalent to 2 mg. culture. In old laboratory strains it lies between 0.5 and 1.5 oesa. Rarely cultures have a higher virulence as in a culture isolated by Lentz which has a virulence of $\frac{1}{100}$ to $\frac{1}{200}$ oesa (Kutscher). Infection with *Bacillus typhosus* does not occur among animals, man alone being sensitive to the natural disease. Attempts to produce typhoid fever in animals have been frequent, especially by Gaffky, E. Fraenkel, and Simmonds.[20] The only successful production of typhoid fever in animals with lesions like those in man is that of Metchnikoff and Besredka,[21] who fed chimpanzees on stools of patients with typhoid fever. The animals developed bloody diarrhea with a characteristic fever, their blood gave positive agglutinations, and the bacillus was isolated from the blood-stream. Cultures of the typhoid bacillus from these chimpanzees were somewhat altered in their properties and would now produce a disease in champanzees which, for all practical purposes, was identical with typhoid fever in man.

**Toxins.**—Attempts to obtain toxic materials from the typhoid bacillus have been frequent, especially stimulated by the manifest intoxication exhibited by many patients. The original efforts to obtain true extracellular toxins were without success, and it has since been maintained that this organism has only an intracellular toxin or endotoxin. Such toxic materials were obtained from the typhoid bacillus in several ways. Thus Sanarelli obtained them by employing old macerated cultures. Martin by killing the organisms by chloroform and heat, MacFadyen and Rowland by freezing with liquid air and grinding, Besredka by drying and grinding with sodium chlorid, Conradi by autolysis, and Vaughan by splitting the organisms by alkali-alcohol. These extracts produce symptoms and lesions in animals, but they are not specific for the typhoid bacillus. According to Gay,[22] the work of Aronson,[23] Arima,[24] Meyer and Bergdell,[25] Kraus and Stenizer,[26] and Yamanouchi[27] must be accepted as proving that true exotoxins are produced by the typhoid bacillus. Antitoxins against the typhoid endotoxin have been produced by Besredka, and this will also neutralize the exotoxin (Arima Stenizer). The typhoid bacillus produces true hemotoxins. Filtrates of sugar-free broth cultures of the organism are hemolytic (Castellani, Levy, and Levy). Antibodies to them have not been produced in animals.

**Distribution.**—During life the typhoid bacillus may be obtained from a variety of locations by the improved technic now in vogue for its isolation and recognition. It was first isolated from the stools by Pfeiffer[28] and E. Fraenkel and Simmonds and is now obtained with great regularity, especially when the stools are thin or fluid. By the use of the v. Drigalski-Conradi medium Kruse[29] obtained it in 68 per cent. of his cases. It may also be obtained on Endo-plates and on Loeffler's malachite green. Peabody and Pratt[30] find that malachite green broth is very valuable for this purpose, while Comradi[31] recommends media containing brilliant green in combination with picric acid. It is best isolated from stools on media containing substances inhibiting *Bacillus coli*, although we have no true enrichment method such as we possess for the cholera vibrio (Kutscher).

From the circulating blood it was first isolated by Castellani,[32] Schottmüller,[33] and Conradi, and it is now obtained with considerable frequency. The blood may be transferred directly to broth or planted on some of the differential media. The blood must usually be diluted, to avoid its bactericidal action (Neufeld[34]). With modern methods the number of successful isolations has become very large. According to Gay blood cultures are positive in 75 per cent. of the cases. The earlier the blood is examined in the course of the disease the larger the number of successful results.

*Bacillus typhosus* has also been cultivated from rose spots by Neufeld. It is present in the urine in cystitis, and in many instances when evidences of cystitis are lacking. The urine may be clear and contain microscopically only a few leukocytes and a small quantity of albumin. The typhoid bacilli when present are usually in large numbers in pure cultures. Konjajoff[35] and Neumann[36] state that small areas of inflammation may be found in the kidneys in these cases even if no well-marked nephritis exists. In typhoid fever with pneumonia the bacillus has been isolated from the hemorrhagic sputum by Glaser[37] and v. Drigalski among others. It has been cultivated from the swollen lymph follicles of the epiglottis by Schultz, from the ulcers in angina in typhoid fever by Bendix[38] and Blum,[39] from the tonsils by v. Drigalski and Kathe.

At autopsy on patients dead of typhoid fever the organism is found widely distributed. It is especially abundant in the spleen, the lymphoid follicles of the intestine, the mesenteric glands, and may be obtained regularly from the liver and kidney. It is present in the gall-bladder with and without cholecystitis. The organism may persist for years in the gall-bladder as in the case reported by Hunner where the attack occurred twenty years previously. It is often found in the heart's blood and in the circulating blood. It is present occasionally in the spinal fluid (Jemma, Guinon) and regularly in the bone-marrow and pericardium.

It is also found in the complications of typhoid as in meningitis (Southard and Richards), bone abscesses as in the tibia (Valentin), subcutaneous and muscular abscesses (Pratt), ulceration of the vulva and vagina (Lartigau), inflammation of the testicle (Tavel) in inflamed seminal vesicles (Marcheldon and Pick), in Bartholin's glands (Takaki and Werner), in prostatitis (Richardson and Pick), in ovarian cysts (Werth), in salpingitis (Galliard and Chaput), in subphrenic abscesses (A. Fraenkel), in liver abscesses (Perthes), in abscesses in the spleen (Roux), in the parotid gland (Jonowski), in the thyroid (Colzi-Tavel), in a thrombus of the femoral vein (Haushalter).

**Survival.**—The typhoid bacillus does not survive outside the body except for brief periods. It has been obtained from spring water by Kübler and Neufeld, Hankin,[40] v. Drigalski,[41] and Conradi, from tap water by v. Jaksch and Rau,[42] Tavel,[43] Gärtner,[44] and Noetel.[45] It was also obtained by Levy and Keyser[46] from the contents of a cement privy vault five months after deposition. It has not been obtained from the many articles of food, such as milk, oysters, and vegetables, which on epidemiological grounds are definitely associated with the distribution of the disease.

**Immune Reactions.**—After an attack of typhoid fever a number of substances appear in the patient's serum. The most important of these is the agglutinin upon which the Widal reaction is based. Others are bacteriolysins of Pfeiffer and Kolle, opsonins of Wright, conglutinins of Bordet and Gay, precipitins of Kraus. Various other reactions chiefly of diagnostic purpose have been described, such as Pflaunder's thread reaction (Mandelbaum), the meiostagmin reaction (Acoli), the ophthalmo-reaction (Chantmesse), the skin reaction (Wolff-Eisner), anaphylaxis (Wolff-Eisner, Kraus, and Stenitzer). Following the pioneer work of Wright vaccination against typhoid fever has come to be regarded as our most important single measure of defence against the disease. Various types of vaccines have been employed and striking results obtained in the diminution of typhoid fever in collected groups of individuals, as in armies. Less successful results have been obtained in civilian population.

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18. Petruschky: Ztschr. f. Hyg., 1912, xii, 261.
19. Pfeiffer and Kolle: Ztschr. f. Hyg., 1896, xxi, 203.
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21. Metchnikoff and Besredka: Ann. de l'Inst. Pasteur, 1911, xxv, 193.
22. Gay: Typhoid Fever. 1918, p. 33.
23. Aronson: Berl. klin. Wchnschr., 1907, xliv, 572.
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31. Conradi: Deutsche med. Wchnschr., 1906, xxxii, 58.
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33. Schottmüller: Deutsche med. Wchnschr., 1900, xxvi, 511.
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34. Neufeld: Ztschr. f. Hyg., 1899, xxx, 498.
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———: Centralbl. f. Bakteriol. [etc.], 1899, vi, 672.
36. Neumann: Berl. klin. Wchnschr., 1890, xxvii, 121.
37. Glaser: Deutsche med. Wchnschr., 1902, xxviii, 772.
38. Bendix: Deutsche med. Wchnschr., 1902, xxviii, 409.
39. Blum: Semaine méd., 1908, xxviii, 37.
40. Hankin: Centralbl. f. Bakteriol. [etc.], 1899, xxvi, 554.
41. v. Drigalski: Centralbl. f. Bakteriol. [etc.], 1904, xxxv, 776.
42. v. Jaksch and Rau: Centralbl. f. Bakteriol. [etc.], 1904, xxxvi, 584.
43. Tavel: Kor-Bl. f. Schweizer Aertze, 1887, p. 390.
44. Gärtner: Klin. Jahrb., 1902, ix, 335.
45. Noetel: Sanitätsber über die Kgl. Preuss. Armee, 1903–04, p. 25.
46. Levy and Keyser: Centralbl. f. Bakteriol. [etc.], 1902, xxxiii, 489.

**Carriers.**—Following an attack of typhoid fever a certain number of individuals become chronic carriers. The proportion of carriers has been variously reported and varies from 1 to 5 per cent. Lentz gives the proportion of carriers at about 4 per cent. Park and Williams found 6 carriers in 140 institution convalescents. Gay[1] has made a very careful compilation and reports that the number of carriers reported by different authors is shown in the following table:

*Percentage of Chronic Typhoid Carriers Found by Various Investigators in a study of Convalescent Cases*

| Author. | Date. | Number of Cases. | Percentage of Carriers Three Months or More. |
|---|---|---|---|
| Lentz | 1905 | 400 | 3 |
| Conradi | 1907 | 400 | 0.5 |
| Klinger | 1907 | 482 | 1.7 |
| Kayser | 1907 | 101 | 3.5 |
| Semple and Greig | 1908 | 86 | 11.6 |
| Park | 1908 | 68 | 5.9 |
| Tsuzuki | 1910 | 51 | 5.8 |
| Bruckner | 1910 | 316 | 3.8 |
| Stokes and Clark | 1916 | 810 | 1.85 |

Carriers have been detected many years after the original attack of typhoid fever and practically all individuals who are carriers three months after an attack of the disease remain carriers during life. In some instances the attack was many years before the detection of the carrier condition as in the instances reported by Bolduan and Noble of forty-six years and by Gregg of fifty-two years. In carriers there is usually some disease of the gall-bladder but pure intestinal carriers have been described by Kraus[2] as associated with chronic intestinal ulcerations. In addition to the individual who discharge typhoid bacilli with the dejecta, a certain number of urinary carriers develop after typhoid fever. Garbat[3] states that typhoid bacillurea lasting two to three months develops in 6.8 per cent. of all typhoid cases. Prigge[4] and Houston and Erwin[5] have described chronic urinary carriers. These are probably individuals with some lesion of the genito-urinary tract.

The recognition of carriers is accomplished by stool and urine examination. Garbat, who has recently made an intensive study of carriers, has employed the duodenal tube with great success in the search.

1. Gay: Typhoid Fever, 1918, p. 122.
2. Kraus: Wien. klin. Wchnschr., 1914, xxvii, 1433.
3. Garbat: J. Am. M. Ass., 1916, lxvii, 1493.
4. Prigge: Klin. Jahrb., 1909–10, xxii, 245.
5. Houston and Erwin: Lancet, 1909, i, 311.

### Dysentery Bacilli

Modern work on epidemic or bacillary dysentery may be said to start with the investigations of Shiga[1] in Japan who traced the etiological relation of the organisms he obtained from the intestine by means of the agglutination reaction. The organism obtained by Shiga had further characteristic morphological and cultural features which differentiated it from any organisms known at that time. Bacilli regarded as identical with the Shiga bacillus were isolated two years later by Kruse[2] in Germany, by Flexner[3] in the Philippine Islands, and by a number of other observers. Practically all these organisms were agglutinated by the patient's serum. With the increasing number of isolations from dysentery more and more attention was paid to differential cultural reactions and it was recognized that whereas the strains obtained by Kruse were identical with those of Shiga, those found by Flexner could be separated by reactions with the carbohydrates and represented a distinct but different type of dysentery bacillus. Other types were soon recognized, especially by American observers such as Strong and Musgrave,[4] Hiss and Russell,[5] Duvall, etc. In the meantime, Kruse had described an organism somewhat different from the Shiga bacillus from cases of so-called asylum dysentery or "pseudo-dysentery." According to Liefmann and Nieter[6] and Dopter[7] the pseudo-dysentery organisms of Kruse are identical with Bacillus Y of Hiss and Russell. We now recognize four main types of dysentery bacilli: those of Shiga, Flexner, Hiss and Russell (Y), and Strong, together with Duval's organisms. These types can be differentiated culturally from each other and respond specifically to immune sera. As has been shown, especially by Schmitz,[8] there may be other types differing serologically.

Following the discovery of these various kinds of dysentery bacilli, attempts were made to obtain toxic products of the nature of endotoxins or true extracellular toxins from them. These attempts have been more or less successful with the Shiga bacillus which apparently produces a true extracellular toxin. It may thus be differentiated from the other dysentery

Fig. 86.—*Bacillus dysenteriæ* Shiga from plain agar, twenty-four hours, stained with gentian-violet.

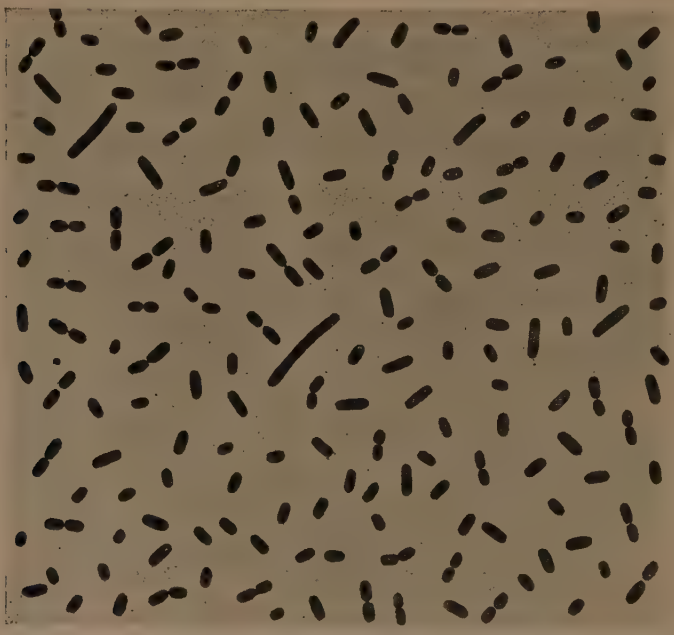

Fig. 87.—*Bacillus dysenteriæ* Shiga from dextrose agar twenty-four hours old, stained with gentian-violet.

bacilli which produce endotoxins but no extracellular toxin. Lentz[9] divides the dysentery bacilli into the toxic dysentery bacillus of Shiga, and the toxic-poor dysentery bacilli of Flexner, Hiss and Russell, and Strong. (See Davidson.[10])

### Bacillus dysenteriæ Shiga

Described by Shiga. Subsequently isolated by bacteriologists in many parts of the world in cases of dysentery, and in some of the more severe cases of summer diarrhea, especially in America.

**Morphology.**—Plump, short bacilli with round ends, usually somewhat shorter and thicker than *Bacillus typhosus*. On plain agar in twenty-four hours, it measures about 0.4 $\mu$ in breadth by 0.5 to 1.5 $\mu$ in length. Threads formed somewhat rarely except in media to which strong potassium solution is added (Peju and Rajat[11]). Mühlmann[12] and Bernhardt[13] find that involution forms are produced on strongly alkaline media. In old secondary colonies Winter has described threads and large involution forms with club- and barrel-shaped enlargements. On dextrose agar in twenty-four hours the organisms are swollen, measuring 0.6 to 0.7 $\mu$ in breadth by 2.5 to 3.5 $\mu$ in length. Many forms are much longer, measuring 5 to 7 or 8 $\mu$ in length.

No spores. Non-motile. No flagella.

**Staining Reactions.**—Stains easily, but irregularly by the ordinary anilin dyes, gentian-violet, methylene-blue, dilute carbolfuchsin. Some organisms take the stain deeply, others lightly. With methylene-blue or dilute carbolfuchsin the organisms may show polar staining, especially on potato (Kruse) or from the peritoneum of infected guinea-pigs. Gram-negative. Nakanishi[14] has described irregular nuclear structures.

**Cultivation.**—Isolated from the stools best by the methods in vogue for obtaining *Bacillus typhosus*, selective media being used in which the colonies may be differentiated easily from colonies of *Bacillus coli* or other intestinal bacteria. Growth good on neutral, slightly acid or slightly alkaline media under aërobic and anaërobic conditions. Growth at temperatures ranging from 22° to 37° C. No growth below 6° C. Peju and Rajat recommend the addition of 4 drops of an aqueous solution of potassium iodid to a tube of broth to favor the development, 8 drops inhibiting it.

**Cultural Characters.**—*Plain Agar Plates.*—Surface colonies in twenty-four hours small, delicate, round and smooth, measuring $\frac{1}{2}$ to 1 mm. in diameter. They are usually pale and translucent, sometimes almost bluish in color. Under low power they are granular with small dark central nuclei and thin margins, edges entire. Deep colonies punctiform. Under low power ovals and disks. The surface colonies may increase rapidly in size and in two to three days are sometimes 0.5 cm. in diameter, flat and smooth, with crenated edges. Generally however, they remain smaller, 2 to 3 mm. in size. Deep colonies increase somewhat and after several days may be $\frac{1}{2}$ mm. in diameter.

*Plain Agar Slant.*—Delicate flat growth in twenty-four hours spreading uniformly over the surface. The growth increases in size especially in the lower part of the tube and may cause considerable turbidity in the water of condensation. Growth remains thin and delicate even in old cultures.

*Plain Agar Stab.*—Delicate line and some surface growth about the point of puncture in twenty-four hours. The line and surface growth increases somewhat in three to four days but remains delicate even in old cultures.

*Glucose Litmus Agar Plates.*—Surface colonies in twenty-four hours delicate and flat, 2 to 3 mm. in diameter. Under low power rather opaque, centers dark, margins thin, edges entire. Deep colonies delicate, $\frac{1}{2}$ mm. in diameter. Under low power dark ovals or biconvex disks. Agar highly acid. Surface colonies increase somewhat in size and at the end of five to six days may be 3 to 5 mm. in size. The deep colonies remain small and delicate. Acid reaction usually permanent. No gas.

*Glucose Litmus Agar Slants.*—Moderate growth in twenty-four hours especially in the lower portions of the tube with an acid reaction. Growth increases somewhat in extent spreading over the agar surface in five to six days. Reaction remains highly acid.

*Glucose Litmus Agar Stab.*—Good line and surface growth in twenty-four to forty-eight hours. Litmus reduced in the depths where anaërobic conditions prevail, not above where there is free access of atmospheric oxygen. Reaction acid.

*Lactose Litmus Agar* (v. Drigalski and Conradi).—Homogeneous, round, dew-drop colonies in sixteen to twenty-four hours, 1 cm. in diameter, slightly milky, turbid by transmitted light. The background of agar remains blue.

*Gelatin Plates.*—Colonies appear in two to three days, small, delicate, grayish, transparent, $\frac{1}{2}$ to 1 mm. in diameter. Under low power they are translucent and granular with irregular, wavy, jagged borders often resembling vine leaves. The colonies do not increase much in size, but secondary or daughter colonies, especially emphasized by Winter, appear about the original colonies and the entire mass of colonies may measure 4 to 6 mm. in size. Old colonies

slightly larger, gray, and delicate. Under low power granular with thread-like processes. No liquefaction. According to Lentz the same type of colony appears in 10, 15 and 20 per cent. gelatin.

*Gelatin Stab.*—Multiplication slow, a delicate line growth and a slight surface growth appearing in two to three days. Increase of growth moderate and at the end of nine to ten days the line growth is still delicate with a moderate superficial expansion at the point of puncture. No liquefaction. On Piorkowski's urine gelatin the colonies develop processes like those seen in colonies of *Bacillus typhosus* (Rosenthal).

*Blood-serum.*—Delicate, slightly yellowish expansion in twenty-four hours, increased somewhat in two to three days. In cultures ten to eleven days old the growth is delicate, flat, and often cream-colored.

*Potato.*—Development varies with the reaction of the potato. It may fail almost entirely on acid potato, being barely visible as a whitish expansion after eight to ten days. On alkaline potato it is more abundant, somewhat yellowish-brown at the same period.

*Broth.*—Turbidity and sediment in twenty-four hours, no scum. Turbidity and sediment increase slightly in four to five days, remaining moderate even in old cultures. Broth does not clear, sediment whitish. Films develop on the surface especially in toxin-producing strains.

*Peptone.*—Faint turbidity and sediment in twenty-four hours. No scum. Turbidity and sediment increase slightly in four to five days, but remain delicate even in old cultures. Sediment usually whitish. Peptone does not clear. In toxin-producing strains delicate films may form on the surface.

*Litmus Milk.*—Faint acidity in twenty-four hours, slightly increased in forty-eight hours. In four to six days an alkaline cream ring may appear on the surface, the reaction in the depths remaining acid. The acidity is usually permanent but not enough to coagulate the milk even on heating.

*Petruschky's Milk-whey.*—Slight acidity like that produced by *Bacillus typhosus*. Whey remains clear.

*Dextrose Fermentation Tube.*—Turbidity in bowl and arm, whitish sediment. No scum. Reaction acid in three days. In *saccharose* and *lactose* the growth remains limited to the bowl, the arm clear with a good line of demarcation. No acid

*Chemical Products.*—The Shiga bacillus reduces nitrates to nitrites but does not form indol. It produces acetic, succinic and formic acid from glucose and from glycerin with a trace of propionic acid from the latter. Cultures and dysentery stools have a characteristic seminiferous odor referred by Winter[15] to trimethylamin. Old sealed agar cultures have a fecal odor.

**Resistance.**—Cultures remain alive for considerable periods and need be transferred only every three to four weeks. Martini has reported living organisms after three years in sealed agar cultures. According to Lentz well-stained round bodies, like the bodies described by Galli-Valerio in plague, are found in dysentery cultures a year old. When protected from drying in air-exhausted spaces, the protoplasm of the organism apparently contracts to small balls or spheres and retains its vitality. Destroyed easily by heat, 60° C. in ten minutes (Kolle, Rosenau). Killed rapidly by disinfectants, in 0.5 per cent. carbolic acid in six hours, in 1 per cent. in thirty minutes. Destroyed instantly in 5 per cent. carbolic acid and in 1 : 20,000 sublimate solution. Killed in a few minutes by dilute alcohol (5 to 10 per cent.). According to Kolle, formaldehyd 1:40,000 prevents their development in milk. Destroyed and dissolved by antiformin (Uhlenhuth and Xylander). Killed by direct sunlight in thirty minutes (Shiga). Resists the cold of winter two months (Schmidt). In feces the organisms die out rapidly; in forty-eight hours according to Kruse. Found viable eight days on the shirts of dysentery patients (Rosculet), three weeks on clothing (Blackham). Die out in milk, butter, cheese, and water in eight days (Pfuhl). Survive ten to twelve days in sterile distilled water, three days in sterilized tap water (Dombrowski). Live twelve days in sand, seventeen days dried on linen (Pfuhl), several months in moist garden earth and on moist linen (Kruse). Found by Rosenthal up to eleven days on artificially infected fruits and vegetables. Not obtained by Dombrowski after three days on bread and potatoes or dried bread crumbs.

**Pathogenic Action.**—Very virulent to ordinary laboratory animals by subcutaneous, intravenous and intraperitoneal injection of living and killed cultures. Rabbits with large doses develop fever, diarrhea with blood and mucus, rapid collapse and die in a short time. With intravenous inoculation of living organisms, $\frac{1}{20}$ to $\frac{1}{50}$ of an oesa, kills rabbits of 1.5 to 2 kilograms in weight. Fatal dose somewhat larger by subcutaneous administration. With killed cultures, $\frac{1}{3}$ of an oesa introduced subcutaneously kills rabbits, $\frac{1}{10}$ of an oesa when introduced into the veins. At autopsy the animals show a hyperemia of the lungs, kidneys, and entire intestinal tract, with a swelling of the lymph-follicles and Peyer's patches of the small intestine. The bacilli may be isolated from the blood and organs, especially the spleen. With small doses the animals develop a chronic intoxication and may survive some time. At autopsy the organisms are not found in the blood or organs. The mucous membrane of the intestinal tract shows an inflammatory thickening with a necrosis of the epithelium and ulcers in the large intestine. Guinea-pigs are more resistant than rabbits. One-third of an oesa of a living culture being necessary to kill animals of 200 gram weight. Doses of $\frac{1}{2}$ oesa of killed cultures do not usually kill guinea-pigs, larger quantities being required. White mice are more susceptible

than rabbits or guinea-pigs, succumbing to $\frac{1}{100}$ of an oesa injected beneath the skin. Gay[16] has found that large animals (dogs, goats, asses, and horses) are very sensitive to the Shiga bacillus. Attempts to produce dysentery by the Shiga bacillus have been generally fruitless. After feeding whole cultures to young cats and dogs Shiga observed numerous diarrheal stools which contained the dysentery bacillus, but the animals had no lesions of dysentery. According to Firth true dysentery may be produced in apes, *Macacus rhesus*, by feeding. In fasting rabbits with the gastric acidity neutralized, given opium to control the intestinal movements (1 g. of tincture per 200 gram weight), Kazarinow[17] found in one instance a fatal disease which seemed to resemble dysentery in man, both clinically and pathogenically. By cultivation in human and animal bile, growth and virulence are diminished (Ottolenghi).

**Toxins.**—The Shiga bacillus produces an endocellular and an extracellular toxin. The endotoxin was first observed by Conradi,[18] Neisser, and Shiga.[19] Conradi made an autolysate of a twenty-four-hour culture, filtered it, and concentrated it "in vacuo" to $\frac{1}{50}$ to $\frac{1}{10}$ of its volume. Intravenous injection of 0.1 c.c. killed rabbits of $2\frac{1}{2}$ to 3 kilos in forty-eight hours. The symptoms resembled those found with the injection of living cultures. Similar results were obtained by Neisser and Shiga, Vaillard and Dopter,[20] Besredka,[21] Flexner and Sweet,[22] and others. Flexner and Sweet obtained an autolysate of which $\frac{1}{100}$ c.c. was fatal to rabbits by intravenous inoculation. Besredka's final fluid had a fatal dose of $\frac{1}{80}$ c.c. for 1800-gram

Fig. 88.—*Bacillus dysenteriæ* Flexner from plain agar twenty-four hours old, stained with gentian-violet.

rabbits and for white rats in two to three days, while white mice succumbed to 0.0003 to 0.0006 c.c. by intraperitoneal inoculation. Extracellular toxins were first observed by Rosenthal,[23] Todd,[24] and Kraus.[25] Rosenthal found them in cultures in Martin's broth three to four weeks old, 0.3 to 0.1 c.c. being a fatal dose for a 2-kilogram rabbit. Todd precipitated the toxin from cultures in Martin's broth by ammonium sulphate, his dried toxin having a fatal dose of 0.002 gr. for a 1500-gram rabbit. Kraus found similar toxins in the filtrates from cultures in strongly alkaline broth ten to twelve days old, precipitating them with ammonium sulphate and alcohol and finally obtaining them in the form of a dry powder. This toxin produces the same clinical symptoms and pathological changes in animals as are found with living or killed cultures or the endotoxin. It is found only in those strains which grown on the surface of very alkaline fluid media, with free access of oxygen. It retains its strength for months when dried, resists heating to 70° C. in one hour, but is destroyed at 81° C. It resists the action of disinfectants like 2 per cent. formalin in the dark, and is slowly destroyed by it in the light. Not digested by trypsin or pepsin. This toxic substance is regarded by many authors, Kraus and Doerr,[26] Kolle, etc., as a true extracellular toxin distinct from the endotoxin of the organism. Recently Olitsky and Kligler[27] have obtained an exotoxin from the Shiga bacillus by growing

the organisms in alkaline egg broth, and an endotoxin by washing off the organisms from agar growths in salt solution, incubating and filtering. The exotoxin produces severe nerve lesions and paralysis in rabbits, the exotoxin has no such action, producing diarrhea and loss of weight.

### BACILLUS DYSENTERIÆ Flexner

Isolated by Flexner from cases of dysentery in the Philippine Islands. It differs somewhat in cultural features from the Shiga bacillus and can be distinguished from it by serological reactions.

Morphologically the Flexner bacillus is about the same width as the Shiga bacillus but the majority of the individuals are somewhat longer, measuring frequently 2 to 4 $\mu$ in length. Larger forms are common. Culturally the growths are usually somewhat more vigorous. Best growth at 35° to 37° C. *Colonies* on *agar* like those of Shiga. On *glucose litmus agar plates* a permanent acidity is produced. *Gelatin colonies* are apt to be button-shaped, round, and

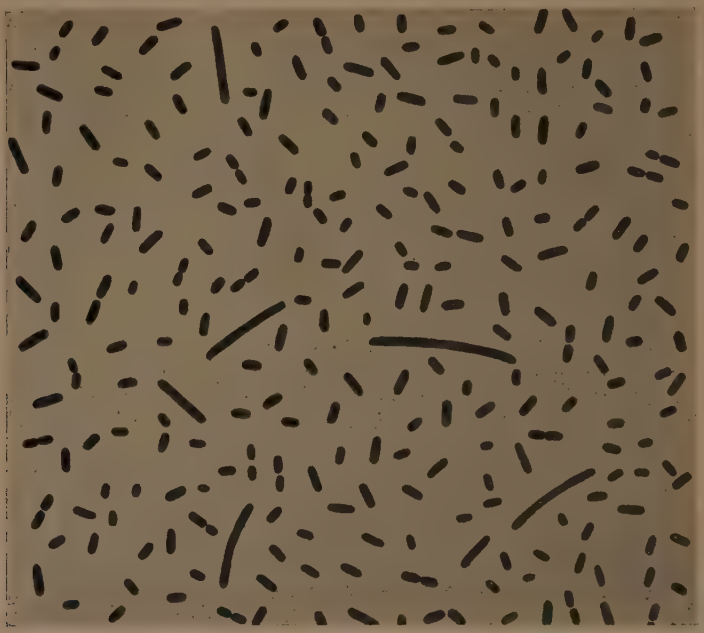

Fig. 89.—*Bacillus dysenteriæ* Flexner from dextrose agar twenty-four hours old, stained with gentian-violet.

raised. Vine-leaf colonies, like those of *Bacillus typhosus* and Shiga, more rarely produced. Films are not produced on the surface of *fluid media*. *Milk* faintly acid at first, then neutral or slightly alkaline. Colonies on v. Drigalski-Conradi medium like those of Shiga. Ferments dextrose, mannite, and maltose with acid production, not saccharose, lactose, or dextrin. Grows well in human bile and in a concentrated oxygen atmosphere (90 per cent.).

Reduces nitrates to nitrites. Some strains produce indol.

**Resistance** to deleterious influences usually greater than that of Shiga. Cultures somewhat easier to maintain artificially. Dies rapidly at 50° to 60° C. (Frost and Swenson).

**Pathogenic action** like that of the Shiga bacillus except that the virulence for laboratory animals is not so high, larger doses being required to kill. Rabbits withstood $\frac{1}{2}$ oesa of the living organism introduced intravenously. Guinea-pigs still more resistant, succumbing to 2 to 5 oesas of living organisms and to 1 to 2 agar cultures if killed. According to Flexner the introduction by mouth produces enteritis in dogs.

The bacterial cell substance is slightly poisonous from an endotoxin. Exotoxins produced rarely and only in small quantity. Kraus and Doerr claim that a weak toxin is occasionally present in filtrates from broth cultures. Kolle, Helle, and de Mestral have obtained them from agar cultures.

### Bacillus dysenteriæ Y of Hiss and Russell

Isolated by Hiss and Russell from a case of fatal diarrhea in a child in America.

Resembles the Shiga bacillus morphologically and culturally. Ferments dextrose and mannite, but not maltose, saccharose, lactose, or dextrin. Colonies on *lactose litmus agar* with or without crystal violet are 2 to 3 mm. in diameter, with clearly marked, jagged, irregular edges. They sometimes show a more reddish-violet color than Shiga and Flexner which are always blue. According to Winter, *lactose* is slightly attacked, but this has not been confirmed. *Milk* first acid then neutral or alkaline. Resistance greater than that of Shiga. Winter found them in sterilized tap water fifteen days after artificial introduction of large quantities in non-sterilized tap water nine days; in dysentery stools up to nine days; on articles of clothing, dried, up to one hundred and fifty days. Killed by direct sunlight in various times up to ten hours.

Pathogenic action like that of Shiga, but not so virulent. Rabbits stand $\frac{1}{2}$ oesa of living agar culture introduced intravenously. Guinea-pigs succumb to 2 to 5 oesas of living organisms; $\frac{1}{2}$ of an agar culture of killed organisms. Mice are sensitive, succumbing to $\frac{1}{100}$ of an oesa of a living culture in twenty-four hours (Hilgermann). Large animals also sensitive. Thus Baermann and Schüffner[28] killed a goat with a single injection of $\frac{1}{20}$ of an oesa, the animal living only eight to ten hours. By feeding apes, *Macacus rhesus*, Bernhardt has produced a condition resembling true dysentery.

### Bacillus dysenteriæ Strong and Musgrave

Isolated by Strong and Musgrave from cases of dysentery in the Philippines. Resembles the Flexner bacillus somewhat more than the Shiga bacillus. *Gelatin* surface colonies vine-leaf in character. Colonies on *litmus lactose agar*, with or without crystal violet, round and blue. Ferments dextrose, mannite, saccharose, rhamnose and raffinose, not maltose. Virulence like that of Flexner. Produces endotoxin, no exotoxin.

### The Schmitz dysentery bacillus

Isolated by Schmitz from cases of dysentery in Roumania and Salonika. Subsequently found among the British troops in Macedonia. Culturally and morphologically a member of the dysentery bacillus group but different serologically. Growth on media somewhat heavier than that of Shiga, surface colonies somewhat larger. *Milk* at first acid than alkaline. *Gelatin* colonies vine-leaf in character.

Ferments dextrose, mannite, rhamnose, and maltose, but not saccharose or lactose.

### Immune Reactions in dysentery

Agglutinins are found in patients with dysentery against the specific agent of the disease and powerful agglutinins may be produced by the immunization of animals which serve to differentiate the principal types of dysentery bacilli and to split up these types into many subtypes. Precipitins are also produced in immune animals as well as bactericidal and bacteriotropic substances, opsonins, and complement-fixing substances. Antitoxic sera have been employed without marked success in the treatment of dysentery. Vaccination has also been tried out on a large scale, the results, in some instances, being favorable.

1. Shiga: Centralbl. f. Bakteriol. [etc.], 1898, xxiii, 599; 1898, xxiv, 817; 870; 913.
2. Kruse: Deutsche med. Wchnschr., 1900, xxvi, 637.
3. Flexner: Centralbl. f. Bakteriol. [etc.], 1900, xxviii, 625. *Ibid.*, 1901, xxx, 449.
4. Strong and Musgrave: Rep. Surg. Gen. Army, Washington, 1900.
5. Hiss and Russell: Med. News, 1903, lxxxii, 289.
   Hiss: J. Med. Research, 1904–05, viii, 1.
6. Liefmann and Nieter: München. med. Wchnschr., 1906, liii, 2, 2097.
7. Dopter: Bull. de l'Inst. Pasteur, 1906, Nos. 1 and 2.
8. Schmitz: München. med. Wchnschr., 1917, lxiv, 1571.
   ———: Centralbl. f. Bakteriol. [etc.], 1919, lxxxiii, 1, 108, 210.
9. Lentz: Dysenterie. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, iii, 899.
10. Davidson: J. Exper. M., 1920, xxxii, 651.

11. Peju and Rajat: Compt. rend. Soc. de biol., 1906, i, 1013.
12. Mühlmann: Arch. f. Hyg., 1910, lxix, 401.
13. Bernhardt: Ztschr. f. Hyg., 1912, lxxi, 229.
14. Nakanishi: Centralbl. f. Bakteriol. [etc.], 1901, xxx, 97.
15. Winter: Ztschr. f. Hyg., 1912, lxx, 273.
16. Gay: Univ. Penn. M. Bull., 1902, xv, 307.
17. Kazarinow: Arch. f. Hyg., 1904, l, 66.
18. Conradi: Deutsche med. Wchnschr., 1903, xxix, 26.
19. Neisser and Shiga: Deutsche med. Wchnschr., 1903, xxix, 61.
20. Vaillard and Dopter: Ann. de l'Inst. Pasteur, 1903, xvii, 463. *Ibid.*, 1905, xix, 353, 753.
21. Besredka: Ann. de l'Inst. Pasteur., 1906, xx, 304. *Ibid.*, 1919, xxxiii, 301.
22. Flexner and Sweet: J. Exper. M., 1906, viii, 514.
23. Rosenthal: Sekt. f. Bakt. der Kaisserl. Gesellsch. für Naturkinde. Moskau, Feb. 1, 1903.
24. Todd: J. Hyg., 1904, iv, 480.
25. Kraus: Monatschr. f. Gsndhtspflg., 1904, No. 11.
26. Kraus and Doerr: Wien. klin. Wchnschr., 1905, xviii, 158.
Doerr: Centralbl. f. Bakteriol. [etc.], 1903, xxxiv, 385.
27. Olitsky and Kligler: J. Exper. M., 1920, xxxi, 19.
28. Baermann and Schüffner: München. med. Wchnschr., 1909, lvi, 1, 384.

### Bacillus alkalescens Andrewes

A non-pathogenic organism, described by Andrewes,[1] like the dysentery bacillus of Flexner. It produces alkali with much vigor in litmus milk which turns dark blue in seven to ten days, then slaty like a milk culture of *Bacillus fecalis alcaligenes*. Acid, but no gas from glucose, maltose, mannite, and dulcite, none from lactose and saccharose. Indol reaction positive.

Kendall and Day[2] have proved that *Bacillus alkalescens* brings about a vigorous change in reaction in plain sugar-free gelatin toward the alkaline side of neutrality. There is a considerable deamination (ammonia formation) and a distinct reduction in amino-nitrogen, but the reaction becomes decidedly alkaline as well. In glucose medium there is an energetic increase of protein nitrogen at the expense of the carbohydrate with a sparing of the protein.

1. Andrewes: Lancet, 1918, i, 560.
2. Kendall and Day: J. Infect. Dis., 1922, xxx, 248.

### Bacillus dispar Andrewes

The lactose-fermenting organism of the dysentery group originally described by Duval. Named by Andrewes. Litmus milk acidified and clotted slowly, usually in two weeks. Acid in glucose, lactose, maltose, mannite, sometimes in saccharose and sometimes in dulcite. Indol reaction positive. Pathogenic to rabbits.

Relation to dysentery not clearly proven.

Andrewes: Lancet, 1918, i, 560.

### Bacillus ambiguus Andrewes

An organism resembling the dysentery bacillus of Shiga. Produces acid in glucose, and has no action on the disaccharids except for an occasional late reaction with saccharose. Acid with mannite and dulcite. Litmus milk rendered first acid, then neutral or alkaline. Indol positive. Virulent to rabbits in overwhelming doses, but no intestinal ulcers produced. No relation to human disease established.

Andrewes: Lancet, 1918, i, 560.

### Bacillus acidiformans Sternberg

Obtained originally by Sternberg from the human liver at autopsy. Organisms corresponding to Sternberg's description have been found repeatedly by the present author in the dejecta of man and of cattle.

**Morphology.**—Short, stumpy bacilli 1.2 $\mu$ in breadth and 1.5 to 3 $\mu$ in length according to Sternberg, often growing into filaments 5 to 10 $\mu$ long. In our own isolations the organisms were slightly smaller and measured 0.4 to 0.5 $\mu$ in width and 1 to 2 $\mu$ in length. Much enlarged on dextrose agar, measuring 0.8 to 1 $\mu$ in width and 2 to 3.5 $\mu$ in length. Non-motile. No capsules.

**Stained** with the usual anilin dyes. Gram-negative.

**Cultivated** easily on the usual laboratory media both at 22° C. and at 37° C. Aërobic.

*Plain Agar Plates.*—Surface colonies in twenty-four hours, round, regular, uniform, 1 to 2 mm. in diameter. Under low power granular, heaped up in the center, often showing distinct nuclei. Deep colonies pin-point, under low power oval and disk-shaped. In three to four days the surface colonies are much enlarged, 3 to 6 mm. in size, while after ten to twelve days they are large, flat, thin, with irregular margins, $\frac{3}{4}$ cm. in diameter. Deep colonies when well developed are oval and disk-shaped to the naked eye.

*Plain Agar Slant.*—Thick, abundant, moist growth in twenty-four hours, somewhat resembling that of *Bacillus coli*. In three to four days the growth is thicker and more abundant, whitish in color. At the end of ten to twelve days the growth is thick, white, and abundant.

*Plain Agar Stab.*—Abundant line and surface growth in twenty-four hours. The line growth becomes thicker and more abundant, the surface growth thick and spreading.

*Dextrose Agar Plates.*—Surface colonies round, regular, uniform, and flat, measuring $\frac{1}{2}$ to 2 mm. in twenty-four hours. Under low power they show rather dense centers, thin margins, with entire edges. Deep colonies punctiform, under low power ovals and biconvex disks. Agar highly acid. After four to five days the surface colonies are about 2 mm. in diameter and do not increase much beyond this. The deep colonies when older are well-marked ovals. Acidity permanent.

*Dextrose Agar Slant.*—Fairly abundant, white, elevated growth in twenty-four hours, the agar being highly acid. At the end of seven to eight days the growth is considerably thicker and broader, whitish in color. Acidity permanent.

*Gelatin Plates.*—Development slow, colonies appearing in about three days, and varying in size from $\frac{1}{4}$ to 1 mm. Under low power they are round, regular, with thin margins and entire edges. Older colonies are somewhat larger and resemble gelatin of *Bacilli coli*. No liquefaction.

*Gelatin Stab.*—Faint line and surface growth in twenty-four hours, slightly increased in three to four days. No liquefaction.

*Blood-serum.*—Abundant, thick, moist, white, elevated growth in twenty-four hours. In three to four days it is thicker and somewhat broader. No liquefaction.

*Potato.*—Moderate yellowish growth in twenty-four hours, considerably increased in size in three to four days. In old cultures the growth tends to be reddish-yellow.

*Broth.*—Faint turbidity, considerable sediment in twenty-four hours. No scum. Turbidity and sediment increase in older cultures.

*Peptone.*—Faint turbidity and considerable sediment. No scum.

*Litmus Milk.*—Marked acidity in twenty-four hours, increased in three to four days. Coagulation, usually only on heating.

**Smith Fermentation Tubes.**—*Dextrose.*—Turbidity in bowl in twenty-four hours with considerable sediment. No scum. Growth gradually extends up into closed arm. Acidity high. No gas.

*Saccharose.*—Heavy turbidity in bowl and arm. Considerable sediment. Highly acid. No gas.

*Lactose.*—Turbidity in bowl and arm. Considerable sediment. No scum. Highly acid. No gas.

Virulent to rabbits and guinea-pigs by intraperitoneal inoculation, the animals dying of septicemia in twenty-four hours.

**Remarks.**—Some of the reactions described by Sternberg do not appear in the author's cultures. The latter, however, represent a distinct type which corresponds more closely to Sternberg's description than to any other species.

Sternberg: Manual of Bacteriology, 1893, p. 449.

### Bacterium anaërogenes Lembke

Described originally by Lembke[1] from the human dejecta. Named *Bacterium Lembkei* by Migula.[2] Apparently the same species was reported by Castellani[3] as *Bacillus ceylonensis* in 4 cases of a febrile disease in Ceylon. Found by Wilson[4] in the urine in 6 cases of cystitis, and by Swan[5] in human feces.

**Morphology.**—Short, non-motile rods not provided with capsules and not producing spores. Protoplasm free from granules.

**Stained** easily with methylene-blue. Gram-positive according to Lembke, Gram-negative according to Castellani and Swan. Not acid-fast.

**Growth** slow on ordinary media, best at 37° C.

*Agar Plates.*—Surface colonies in twenty-four hours round, bluish, with entire edges and uniform internal structure. Deep colonies punctiform, brownish, under low power homogeneous with entire edges.

*Agar Slant.*—Growth slow, an irregular whitish expansion appearing in forty-eight hours.

*Gelatin Plates.*—Small, whitish colonies in two to three days, under low power yellowish brown with irregular margins. The colonies never become large. No liquefaction.

*Gelatin Stab.*—Faint line and surface growth. No liquefaction. The line growth may be made up of discrete colonies (Lembke).

*Potato.*—Irregular, slightly elevated, brownish growth appearing usually only after several days.

*Broth.*—Faint turbidity. No scum. No odor.

*Milk.*—Acidification, no coagulation.

Acid, no gas in glucose, saccharose, and lactose.

Virulent to guinea-pigs by subcutaneous inoculation of 1 c.c. twenty-four-hour broth cultures (Swan). At autopsy the animals show enteritis, peritonitis, congestion of the liver and lungs, cloudy swelling of the kidneys.

**Remarks.**—It is not entirely certain that the subsequent isolations all represent Lembke's species. The type of organism seems distinct however.

1. Lembke: Arch. f. Hyg., 1896, xxvi, 293.
2. Migula: p. 417.
3. Castellani: J. Hyg., 1907, vii, 1.
4. Wilson: J. Hyg., 1908, viii, 543.
5. Swan: J. Trop. M. and Hyg., 1909, xii, 239.

### Bacterium Group IV

Non-liquefying Gram-negative bacteria producing acid and some gas from certain carbohydrates. Especially virulent to birds. The fowl typhoid group.

*Bacterium sanguinareum* Moore.
" *pullorum* Rettger and Harvey.
" *Rettgeri* Hadley.
" *Pfaffi* Hadley.
" *Jeffersoni* Hadley.

### Bacterium sanguinareum Moore

Isolated by Moore[1] from an infectious disease of the fowl in which blood changes, consisting of the destruction of the red blood-corpuscles and an increase of the white blood-corpuscles, predominated. The lesions are sometimes called leukemia. The organism was regarded as distinct from *Bacillus gallinarum* of Klein but more recent investigations by Hadley[2] point to their identity. Apparently the same organism has been described by Berry and Ernst[3] as *Bacterium pyogenes sanguinareum*, by Pfeiler and Rehse[4] as *Bacterium typhi gallinarum alcalifaciens*. Usually called the organism of fowl typhoid.

**Morphology.**—A non-motile, rod-shaped organism varying somewhat in size according to the medium in which it has developed. In the tissues of fowls and rabbits it is from 1 to 1.3 $\mu$ broad and from 1.2 to 1.8 $\mu$ long (Moore.) The ends are tapering or rounded in cultures and at times the short forms look like cocci. In tissues it is found in small clumps, or in pairs united end to end. Polar granules marked in unstained preparations. In cultures on agar it is more slender than in the tissues. Short chains appear in old bouillon cultures. No spores or vacuoles. Involution forms common. In cultures studied by the present author the organisms cultivated on plain agar were about 0.5 to 0.7 $\mu$ in breadth, and 1 to 3 $\mu$ in length. On dextrose agar they were swollen and almost oval, 0.7 to 1 $\mu$ in breadth and 1 to 1.5 $\mu$ in length, with bacillary forms measuring 0.7 by 2 $\mu$.

**Stained** easily with ordinary anilin dyes. Gram-negative. Polar granules well-marked.

**Cultivated** without difficulty on ordinary media. Grows at room temperature, at 36° to 41° C., and more slowly at about 13° C. in the ice-box.

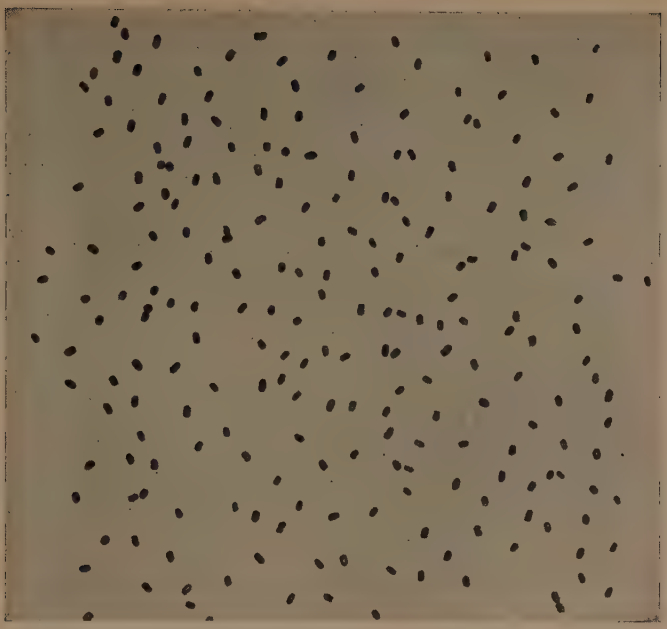

Fig. 90.—*Bacterium sanguinareum* from plain agar twenty-four hours old, stained with gentian-violet.

Fig. 91.—*Bacterium sanguinareum* from dextrose agar, twenty-four hours old, stained with gentian-violet.

**Cultural Characters.**—*Plain Agar Plates.*—Colonies appear in twenty-four hours, small, round, and regular, showing central nuclei and entire margins under low power. They increase slowly in size and are about 2 mm. in diameter; after two to four days somewhat more dense and opaque. Older colonies may be somewhat larger but in general the agar colonies of this organism tend to be small and circumscribed. Agar plates give off a peculiar, penetrating odor (seminiferous?).

*Plain Agar Slant.*—Grayish, glistening expansion in twenty-four to forty-eight hours, somewhat moderate in extent. It resembles the growth of the hog-cholera bacillus. In three to four days it spreads slightly below, after eight to ten days the growth is considerably thicker with heaped up edges.

*Plain Agar Stab.*—Faint line and surface growth in twenty-four hours. The line growth remains delicate while the surface growth becomes a trifle thicker and more elevated after six to eight days.

*Dextrose Agar Plates.*—Superficial colonies appear in twenty-four hours, round, regular, translucent, $\frac{1}{4}$ mm. in diameter, under low power round and regular with entire edges. Deep colonies pin-point, under low power biconvex disks. The colonies remain small and after five to six days those on the surface are only about $\frac{1}{2}$ to 1 mm. in diameter, while the deep colonies appear as well-developed biconvex disks to the naked eye. Agar acid.

*Dextrose Agar Slant.*—Fairly abundant growth in twenty-four hours with the agar definitely acid. The growth increases slowly in breadth and thickness, and the acid reaction tends to diminish. In old cultures the reaction is often alkaline.

*Gelatin Plates.*—Small, grayish, delicate pin-point colonies in two to three days. Under low power they are slightly granular, round, regular with entire edges. Older colonies may be $\frac{1}{4}$ to $\frac{1}{3}$ mm. in diameter. No liquefaction. Smith and TenBroeck[5] state that the surface colonies on gelatin are fleshier and not so leaf-like as those of *Bacillus typhosus*.

*Gelatin Stab.*—Delicate line and surface growth in three to four days, somewhat more abundant in eight to ten days. No liquefaction.

More vigorous growth in alkaline than in acid gelatin (Moore).

*Loeffler's Blood-serum.*—Faint, delicate growth in twenty-four hours becoming slightly more abundant in five to six days.

*Potato.*—Delicate, grayish-yellow growth in twenty-four to forty-eight hours on alkaline potato. After five to six days it becomes more abundant and slightly yellow. No growth on acid potato (Moore).

*Broth.*—Faint turbidity, no scum. Broth cultures less turbid than those of *Bacillus typhosus* except in old shallow cultures (Smith and TenBroeck). Alkaline reaction develops somewhat slowly. After several days growth settles to the bottom as a grayish, friable sediment with a clear supernatant fluid above.

*Peptone Solution.*—Faint turbidity and sediment.

*Litmus Milk.*—Slow development. Slight preliminary acidity, followed in a few days by an alkaline reaction. After about four weeks the milk changes to a clear opalescent fluid, and after six weeks it becomes translucent and strongly alkaline (Moore). The casein is precipitated by a few drops of acetic acid. The fat globules are destroyed, possibly by the alkali. In milk cultures in fermentation tubes the reaction becomes alkaline in the open bulb while with *Bacillus typhosus* it may be acid or alkaline (Smith and TenBroeck).

**Smith Fermentation Tubes.**—*Dextrose.*—Turbidity in bulb and arm in twenty-four hours, slightly increased in two to three days. Reaction acid.

*Saccharose.*—Turbidity in bulb, arm clear. Reaction alkaline.

*Lactose.*—Turbidity in bulb, arm clear. Reaction alkaline.

Smith and TenBroeck[5] find that this organism produces acid in dextrose, saccharose, lactose, maltose, mannite, salicin, and raffinose. Rettger and Koser[6] report acid from dextrose, levulose, galactose, maltose, mannite, glycerin, dulcite, and dextrin. No acid from saccharose, lactose, raffinose, inulin, adonite, and salicin. Some acid in xylose and arabinose (Hadley). Indol positive. Phenol negative. $H_2S$ positive. No Voges-Proskauer. Nitrates reduced to nitrites. Methyl red positive in five-day cultures in maltose broth. Destroyed at 58° C. in ten minutes. Killed by 1 per cent. carbolic acid in five minutes; 0.25 commercial $H_2SO_4$ in ten minutes; by lime water in ten minutes, and by mixtures of 1 part of lime water and 3 parts of distilled water in one hour.

**Pathogenic Action.**—Virulent to fowls, pigeons, rabbits, guinea-pigs, and mice. Large doses required except with intravenous inoculation. Fowls die in three to thirteen days after intravenous inoculation of 0.3 c.c. of a fresh bouillon culture. At autopsy the liver is moderately enlarged, soft and fatty, spleen rarely enlarged. Kidneys marked with yellow lines, due to injection of tubules with urates. Punctiform hemorrhages rarely found in intestinal mucosa, especially in the colon. Heart muscle pale and sprinkled with grayish points. Occasionally tubercle-like nodules in heart and rarely in lungs. Feeding with infected material occasionally fatal to fowls. According to Pfeiler and Rehse infection can be produced in fowls by feeding diseased material and inoculating cultures into muscular tissues. Rats, dogs, and cats show no disturbance after eating infected substances. Rabbits die in three to five days from 0.2 c.c. amounts given subcutaneously. At autopsy there are slight local reactions, necrotic

areas in the liver, an enlarged and discolored spleen. Intravenous injection fatal in eighteen hours to three days. At autopsy the spleen is engorged with blood, liver swollen, with hemorrhages in the intestinal mucosa. Guinea-pigs die in five to eight days from 0.2 to 0.3 c.c. of broth cultures given intraperitoneally. At autopsy the viscera are covered with a grayish, membranous exudate. Subcutaneous injection fatal in six to eight days. Spleen enlarged, dark, friable. Liver pale and usually fatty. White and gray mice die in three to five days from 1 to 2 drops given subcutaneously. Spleen enlarged and discolored. In nearly all inoculated animals the organs contain comparatively few organisms.

The filtrates from cultures are also toxic (Smith and TenBroeck). Thus 2 c.c. of a filtered five-day broth culture kills rabbits acutely. In one-half to one and a half hours the animals appear sick with marked dyspnea and lacrimation. Pupils not contracted. Occasional discharge of feces and urine. Death violent, the animal falling to one side, giving a few sharp cries, kicking violently, or hopping through the air. At autopsy the liver is filled with blood, spleen and kidneys slightly congested with occasional hemorrhages into the gastric mucosa. The lungs are edematous, the heart in diastole. Both intravenous and intraperitoneal inoculation produce this effect, while subcutaneous injection causes only a rise in temperature and a loss of weight. Filtrates are fatal also to dogs by intravenous injection, while in cats they cause rapid and marked drop in temperature with diarrhea. The filtrates have no effect on monkeys (*Macacus rhesus*), pigeons, or white rats. This toxic substance is produced by autolysis of the organism which begins on the second day in broth cultures and occurs also in milk. It is not destroyed at 60° C. nor completely by boiling fifteen minutes. Immunity to this toxin does not develop or is hard to establish. After repeated doses rabbits show a toxic cirrhosis of the liver like that described by Pearce[7] and may be immune to the living organisms.

**Immune Reactions.**—Animals may be immunized without difficulty by living organisms. According to Smith and TenBroeck the organism of fowl typhoid has agglutinative affinities to *Bacillus typhosus*. Thus an immune serum from rabbits treated with either fowl typhoid or human typhoid agglutinates both organisms in the same or nearly the same dilution. The typhoid bacillus will remove from such a serum all the agglutinins for fowl typhoid, but fowl typhoid will not remove all those for human typhoid. In convalescence from typhoid fever in man, agglutinins for fowl typhoid may be present in the serum.

1. Moore: Rep. 12 and 13, Bureau Animal Industry, 1895–96, p. 185.
2. Hadley: Agric. Exp. Sta., Rhode Island State Col., 1918, Bull. 174.
3. Berry and Ernst: J. Med. Research, 1903–04, x, (n. s. v), 402.
4. Pfeiler and Rehse: Mitt. a. d. Kaiser Wilhelms. Inst. f. Laudwirtschaft., in Bromb., 1913, p. 306.
5. Smith and TenBroeck: J. Med. Research, 1914–15, xxxi, (n. s. xxvi), 503; 523; 547.
6. Rettger and Koser: J. Med. Research, 1916–17, xxxv ,(n. s. xxx), 443.
7. Pearce: J. Exper. M., 1906, viii, 64.

### BACTERIUM PULLORUM Rettger and Harvey

Found by Rettger and Harvey[1] in the disease of chicks known as "white diarrhea."

**Morphology.**—Long slender bacilli with slightly rounded ends. They usually occur as single organisms, chains of more than two bacilli being rarely found. The organisms somewhat resemble *Bacillus typhosus* in morphology. In the author's cultures they measure about $\frac{1}{2}$ $\mu$ in thickness and 1 to 2 $\mu$ in length when taken from plain agar twenty-four-hours old. On dextrose agar the organisms are slightly enlarged in forty-eight hours.

Non-motile. No spores.

**Stained** readily with the ordinary anilin dyes. Gram-negative. Not acid-fast with dilute acetic or mineral acids.

*Grows* on ordinary media, best at 36° to 37° C.

**Cultural Characters.**—*Agar Plates.*—Small, white, delicate surface colonies in twenty-four to forty-eight hours. Under low power round, regular, with entire edges. Deep colonies pin-point, under low power ovals and disks. After three to four days' incubation the surface colonies have a diameter of 1 mm. Under low power they are yellow and vary in form from oval and spindle-shaped to round. The surface is usually marked with rosette figures, or what seems to be a lobate nucleus. Occasionally two or three of these markings may be found.

*Agar Slant.*—Growth visible in twenty-four hours like that of the typhoid bacillus. It spreads little and remains delicate, even after prolonged cultivation.

*Agar Stab.*—Delicate line growth and slight surface expansion even in old cultures.

*Glycerin Agar.*—Growth practically the same as on plain agar.

*Dextrose Agar Plates.*—Superficial colonies small and delicate in twenty-four hours, about $\frac{1}{4}$ mm. in size. Under low power they are round, regular, translucent, nucleated, with entire edges. Deep colonies pin-point, under low power biconvex disks. In two to three days the surface colonies are somewhat larger, slightly elevated, $\frac{1}{2}$ mm. in diameter. They seldom increase beyond this size even in old cultures. Agar acid at first, slowly becoming alkaline.

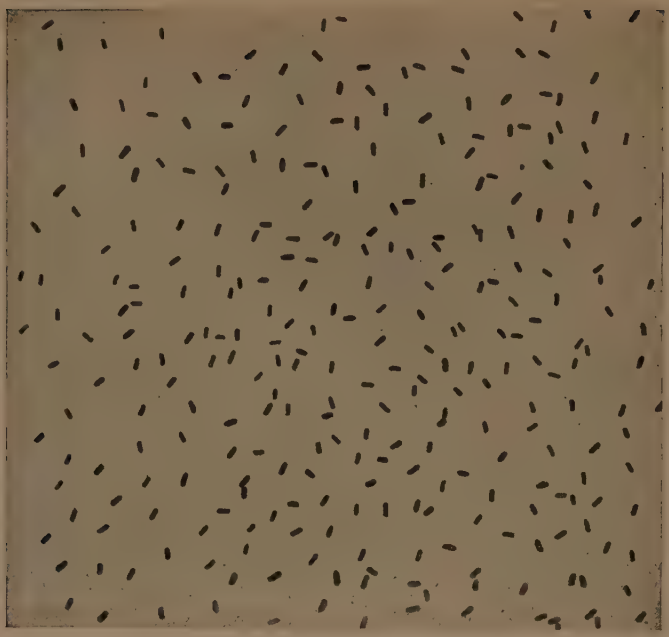

Fig. 92.—*Bacterium pullorum* from plain agar twenty-four hours old, stained with gentian-violet.

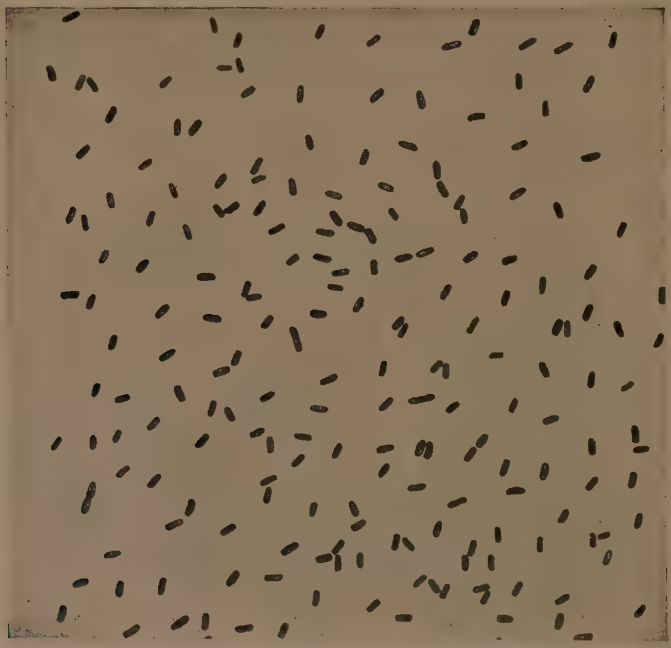

Fig. 93.—*Bacterium pullorum* from dextrose agar twenty-four hours old, stained with gentian-violet.

*Dextrose Agar Slant.*—Thin delicate growth in twenty-four hours, slightly increased in forty-eight hours. Agar acid then becomes alkaline.

*Gelatin Plates.*—Small white colonies in forty-eight hours, under low power round, regular, with entire edges. The colonies remain small and delicate, only under exceptional circumstances developing into characteristic surface colonies which, to a certain extent, resemble the leaf-like colonies of *Bacillus typhosus.*

*Gelatin Stab.*—Delicate growth in forty-eight hours along the entire line of inoculation. it has a distinctly granular appearance and spreads very little on the surface. No liquefaction.

*Potato.*—Growth slow as a narrow almost invisible streak along the line of inoculation.

*Blood-serum.*—Delicate moist whitish growth in twenty-four to forty-eight hours, becoming somewhat elevated in old cultures.

*Broth.*—Faint turbidity in twenty-four to forty-eight hours. No scum, After a few days a scanty sediment may develop, but usually the broth does not clear.

*Peptone.*—Faint turbidity and scanty sediment. No scum.

*Litmus Milk.*—No change within the first forty-eight hours, after which the milk becomes slightly acidified without coagulation. No return to an alkaline reaction. Gas sometimes formed (Smith and TenBroeck[2]).

**Smith Fermentation Tubes.**—*Dextrose.*—Turbidity in bowl and arm in twenty-four hours with a bubble of gas in the arm. This increases to about 1 cm. in forty-eight hours. Reaction acid. Slight absorption of gas by KOH, balance explosive. $\frac{H}{CO_2} = \frac{2\text{–}3}{1}$.

Fermentation also of levulose, galactose, mannite, and glycerin (Rettger and Koser[3]). No action in saccharose, lactose, maltose, raffinose, dextrin, dulcite, inulin, adonite, salicin. Acid in xylose and arabinose (Hadley[4]). Alkali production almost immediate in fermented sugar tubes (Hadley).

Indol not produced. Nitrates not reduced to nitrites. $H_2S$ positive. No Voges-Proskauer reaction. Methyl red negative in five-day cultures in maltose broth. *Killed* by exposure to 56° to 57° C. for fifteen minutes, by corrosive sublimate 1 : 60,000 in two hours at 37° C, by carbolic acid 1 : 200, by formalin 1 : 700.

**Pathogenic** to chicks of different ages by subcutaneous inoculation. The animals develop anorexia and diarrhea, lose flesh and strength, and die in four to eight days. At autopsy the bodies are emaciated, intestines pale and empty, liver pale, spleen, lungs, and kidneys normal. The condition resembles that seen in the natural disease. The organism can be obtained from the internal organs. Subcutaneous inoculation in guinea-pigs produces local infiltration which may soften and become necrotic with cheesy contents. Somewhat similar lesions produced in rabbits, but less advanced. Virulent also to young chick and adult fowls by feeding, somewhat so to young rabbits, guinea-pigs, and kittens.

**Immune Reactions.**—Agglutinins in low dilutions, 1 : 50, in the blood in the natural disease, somewhat higher, 1 : 80, in chicks given injections of dead and living cultures.

Smith and TenBroeck report that the serum from animals immunized to *Bacillus typhosus* agglutinates *Bacterium pullorum* and *Bacterium sanguinareum*, as well as *Bacillus typhosus*, while the serum from animals immunized to *Bacterium pullorum* agglutinates all three organisms. *Bacillus typhosus* removes agglutinins from such sera for both the avian organisms, whereas the latter does not remove the typhosus agglutinins. *Bacterium sanguinareum* and *Bacterium pullorum* thus act the same way in regard to their agglutinins.

Toxins similar to those obtained from filtrates of *Bacterium sanguinareum* can also be obtained from *Bacterium pullorum.*

**Distribution.**—In animals recovering from *Bacterium pullorum* infection, the organisms may remain within the ovary. The eggs of such animals are infected, the chicks hatched from them become diseased and may convey infection to other animals (Rettger).

**Varieties.**—Hadley, Caldwell, and Heath[5] find two types of this organism, A and B. *Bacterium pullorum* A is aërogenic and found only in infections in young stock, or as a latent ovarian infection in adult stock. *Bacterium pullorum* B is anaërogenic and observed only as an agent of active infection in adult stock.

1. Rettger and Harvey: J. Med. Research, 1908, xviii, (n. s. xiii), 277.
2. Smith and TenBroeck: J. Med. Research, 1915–16, xxxi, (n. s. xxvi), 547.
3. Rettger and Koser: J. Med. Research, 1916–17, xxxv (n. s. xxx), 443.
4. Hadley: Agr. Exper. Stat., Rhode Island State College, 1918, Bull., 174.
5. Hadley, Caldwell, and Heath: J. Bact., 1919, iv, 65.

### Bacillus Rettgeri Hadley

Isolated originally by Rettger, who obtained it from a cholera-like epidemic. Named by Hadley, from whom the description is taken.

A very short Gram-negative, non-motile rod; average length 0.5 to 0.8 $\mu$; stains with the ordinary stains, but from cultures does not show either bipolar or peripheral staining. On

*slant agar* and in *gelatin* it shows no distinctive features; the latter is not liquefied. Luxuriant growth on *potato*. Old *broth* cultures do not manifest the viscous ball of sediment characteristic of the fowl-cholera organism. *Litmus milk* is alkalinized in eight days and made translucent, but not changed in consistency. Indol and $H_2S$ are not formed and nitrates are not reduced. The following carbohydrates are fermented with the production of acid: dextrose levulose, galactose, mannose, xylose, (trace), adonite, mannite, and salicin. The following carbohydrates are not fermented: arabinose, raffinose, saccharose, lactose, maltose, dextrin, inulin, dulcite, and erythrite. It differs from *Bacillus avisepticus* in being saccharose-negative. Gas is not formed in any carbohydrate.

Antigenic relation is not shown to any known member of the paratyphoid-enteritidis group, nor to *Bacillus avisepticus;* the homologous antigen is agglutinated only to a slight degree.

Hadley: Agr. Exper. Sta., Rhode Island State College, 1918, Bull. 174, p. 180.

### Bacterium Pfaffi Hadley

Isolated by Pfaff from an epidemic among canaries. Named by Hadley, from whom the description is taken.

A medium-sized, Gram-negative, non-motile rod; average length 1.4 $\mu$, stains with the ordinary dyes, but does not give either peripheral or bipolar staining. On *Slant agar* and in *gelatin* it shows no distinctive features; gelatin is not liquefied. Moderate whitish growth on *potato*. *Litmus milk* is not changed in reaction or consistency after several months. Indol and $H_2S$ are not formed and nitrates are not reduced. The following carbohydrates are fermented with the production of acid, dextrose, levulose, xylose, arabinose, maltose, dextrin, mannite, and salicin. The following are not fermented: lactose, raffinose, saccharose, inulin, adonite, and dulcite. Gas is not formed in any carbohydrate. Antigenic relation is not shown with any known member of the paratyphoid-enteritidis group nor to *Bacillus avisepticus;* the homologous antigen is agglutinated to a slight degree only.

Hadley: Agr. Exper. Sta. Rhode Island State College, 1918, Bull. 174, p. 180.

### Bacterium jeffersoni Hadley

Found by Hadley from an extensive epidemic among poultry. His description follows:

A short, Gram-negative, non-motile rod, average length 0.8 $\mu$. Stains with ordinary stains, but does not show either bipolar or peripheral staining. On *Slant agar* and *gelatin* it shows no distinctive features; the latter is not liquefied. Luxuriant growth on *potato*. In *broth* cultures the growth tends to settle rapidly to the bottom of the tube or flask, leaving the upper layers clear, and producing a heavy and slightly flocculent sediment, quite unlike the viscous sediment of *Bacillus avisepticus*. *Litmus milk* is slightly alkalinized in fourteen days and fully alkalinized in the tenth week, followed by translucency. Indol and $H_2S$ are not formed and nitrates are not reduced. The following carbohydrates are fermented with the production of acid: dextrose, mannose, galactose, levulose, xylose, arabinose, maltose, dextrin, dulcite, and mannite. The following are not significantly fermented: lactose, raffinose, inulin, adonite, erythrite, and salicin. In agglutination tests only a slight antigenic relation is manifested with the fowl typhoid strains, none with *Bacillus avisepticus*. The homologous antigen is agglutinated only slightly.

Hadley: Agr. Exper. Sta. Rhode Island State College, 1918, Bull. 174, p. 181.

### Bacterium Group V

Pathogenic organisms not liquefying gelatin and not producing acid or gas with any carbohydrates.

Type species, *Bacillus alcaligenes* Petruschky.
*Bacterium melitense* Bruce.
" *abortum* Bang.
*Bacillus bronchisepticus* Ferry.
McGowan's organism of dog distemper.
Tartakowsky's bacillus of guinea-pig pneumonia.

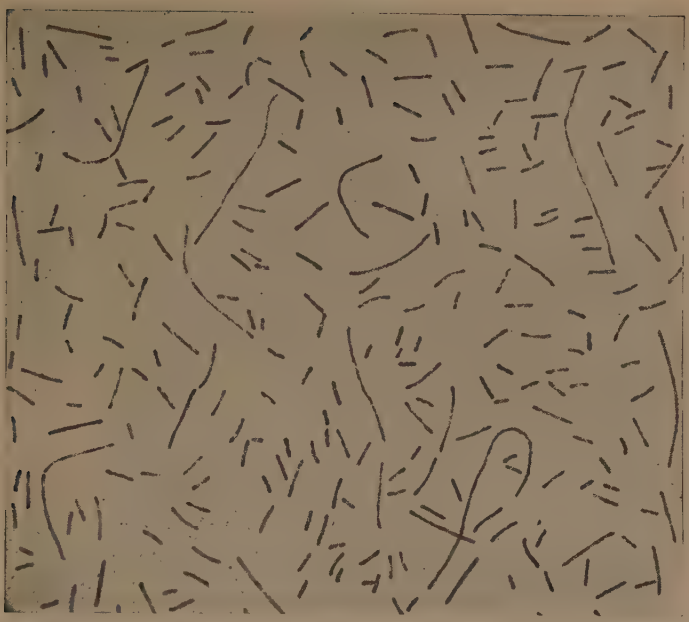

Fig. 94.—*Bacillus alcaligenes* from plain agar forty-eight hours old, stained with gentian-violet.

Fig. 95.—*Bacillus alcaligenes* from dextrose agar forty-eight hours old, stained with gentian-violet.

### BACILLUS ALCALIGENES (Petruschky) Migula

Discovered by Petruschky[1] in spoiled beer and in the feces of man, and called *Bacillus fecalis alcaligenes*. Named by Migula.[2] Fairly common in the dejecta of man and frequently present in the blood and organs of cadavers as a postmortem invader Present description from numerous personal isolations from a variety of sources.

**Morphology.**—Delicate slender bacilli about $\frac{1}{3}$ $\mu$ in breadth and $2\frac{1}{2}$ to $3\frac{1}{2}$ $\mu$ in length. They are frequently longer, measuring 7 to 10 $\mu$ in length. They have a superficial resemblance to *Bacillus typhosus*, but are generally thinner and longer.

**Motility.**—Active, much like that of *Bacillus typhosus*. Frequently the organisms move slowly through the field by a kind of swimming motion. Peritrichous flagella.

**Stains** with the usual anilin dyes, but not deeply. Old organisms tend to show a beaded appearance. Gram-negative.

**Cultivated** easily on ordinary media. Grows well aërobically and anaërobically at both 22° C. and 37° C.

*Plain Agar Plates.*—Surface colonies appear in twenty-four hours, flat, translucent, 1 to 3 mm. in diameter. Under low power delicate, translucent, slightly granular, centers dark, margins thin, edges entire. Deep colonies pin-point, under low power ovals and disks. In three to four days the surface colonies are 4 to 5 mm. in diameter, flat with dentate margins, while the deep colonies are well-developed ovals and disks to the naked eye. The surface colonies often have a peculiar bluish shimmer. Older surface colonies may have a diameter of 6 to 7 mm. and well-defined nuclei. The bottom colonies tend to spread widely between the agar and the glass, often attaining a diameter of 1 cm.

*Plain Agar Slant.*—Moderate smooth, rather thin, and glistening growth in twenty-four hours. In four to five days the growth is abundant and often resembles that of *Bacillus typhosus*. In other cases it is more like *Bacillus coli*, tending to be thick and somewhat heaped up in the center. In eight to ten days the growth is quite abundant, often thick and whitish.

*Plain Agar Stab.*—Moderate line and surface growth in twenty-four hours. The surface growth spreads to the wall of the tube in eight to eleven days, and is flat, glistening, and whitish. Line growth becomes thick and abundant.

*Dextrose Agar Plates.*—Surface colonies in twenty-four hours, round, regular, flat, bluish, 1 to 2 mm. in diameter. Under low power they are flat, thin, often nucleated, denser in the center with thin margins and entire edges. Some colonies show delicate striations. Deep colonies pin-point in twenty-four hours, under low power light ovals and disks. No acid produced, the agar remaining blue. In three to four days the surface colonies are much larger, 4 to 5 mm. in diameter, flat, smooth, round, or irregular. Deep colonies, well-developed ovals and disks, $\frac{1}{2}$ mm. in diameter. In ten to twelve days the surface colonies may have a diameter of $\frac{1}{2}$ to $\frac{3}{4}$ cm.

*Dextrose Agar Slant.*—Moist, thick, white, fairly abundant growth in twenty-four hours. No acid production. After five to six days the growth is much thicker, moist, glistening, and whitish. The agar turns deep blue from alkali production.

*Gelatin Plates.*—Colonies grow slowly, appearing in forty-eight hours as thin and delicate, $\frac{1}{2}$ to 1 mm. in diameter. Under low power they show central masses of granular material surrounded by outlying strands or threads which give a peculiar fuzzy appearance to the colonies. After ten to twelve days the colonies are still small, $\frac{1}{2}$ to 1 mm. in diameter, thin and grayish. Under low power they are now very granular, sometimes with thin entire margins, sometimes with thread-like extensions on the edge. No liquefaction.

*Gelatin Stab.*—Very delicate line and surface growth in twenty-four hours. After four to five days the surface growth may become much more abundant, but the line growth remains faint and delicate. No liquefaction.

*Loeffler's Blood-serum.*—Moderate, slightly yellowish, non-spreading growth in twenty-four hours. In four to five days the growth is somewhat increased in breadth and thickness, cream-colored. No softening of the medium.

*Litmus Milk.*—No macroscopical change in three to four days. After this time the milk becomes deep blue above with an alkaline cream ring. In ten to twelve days the milk is deep blue throughout. No acidity at any time.

*Broth.*—Faint turbidity and sediment in twenty-four hours, no scum. The sediment increases slowly and in eight to ten days there is a considerable deposit of whitish material at the bottom of the tube. The turbidity remains faint and the broth does not clear.

*Potato.*—Moderate, slightly brownish or reddish-brown growth in twenty-four hours. The growth increases in four to five days and tends to develop a well-defined reddish color. Old cultures distinctly brownish-red.

**Smith Fermentation Tubes.**—*Dextrose.*—Turbidity in the bowl in twenty-four hours, no scum, arm clear, good line of demarcation, In three to four days the turbidity is considerably increased, but the arm remains clear with a well-defined line of demarcation. Reaction alkaline.

*Saccharose.*—Growth the same. Reaction alkaline.
*Lactose.*—Growth the same. Reaction alkaline.
Indol not produced.

Pathogenicity to small animals by intraperitoneal inoculation like that of the typhoid bacillus. The organisms multiply and the infection can be carried from animal to animal. When introduced subcutaneously the organisms die rapidly without producing infection. Not agglutinated by *Bacillus typhosus* serum.

1. Petruschky: Centralbl. f. Bakteriol. [etc.], 1896, xix, 187.
2. Migula: p. 737.

Fig. 96.—*Bacterium melitense* from broth culture forty-eight hours old, stained with carbol-fuchsin.

### BACTERIUM MELITENSE Bruce

Obtained originally by Bruce[1] from Malta or Mediterranean fever, subsequently by Hughes.[2] Known also as *Micrococcus melitensis* and *Bacillus melitensis*. An agglutination of the organisms with the serum of the patient was first observed by Wright. The etiology and the epidemiology of the disease was worked out completely by the British Commission. The natural disease is practically limited to the islands of the Mediterranean but a few cases have been reported from America, chiefly from Texas.

**Morphology.**—A very minute coccus measuring when unstained about 0.4 $\mu$ in diameter or a short oval bacillus measuring 0.3 by 0.4 $\mu$ in dimensions. The organisms occur as single elements, pairs, end-to-end, and short chains. In stained material they appear smaller, about $\frac{1}{3}$ of a micron according to Bruce. On plain agar forty-eight hours old they measure 0.3 $\mu$ by 0.5 $\mu$. In older cultures, 1 to two weeks, chains of 10 to 14 elements are found. Old gelatin cultures reveal bacillary forms 2 to 4 times as long as broad. Eyre[3] believes that these long organisms are either dividing cocci or involution forms. In old cultures pear-shaped and oval elements occur. In milk the organisms appear as cocci larger than on any other medium except potato, where they appear as large cocci, grow in chains and produce involution forms.

*Stains* well with methylene-blue, fuchsin, and neutral red. Gram-negative.

**Cultivated** fairly easily. Optimum temperature 37° C. Slow development at room temperature and at 42° C. No growth below 6° C. or over 45° C. Facultative anaërobe. Op-

timum reaction for media +2 on Eyre's scale. Grows well on media of +10 reaction, extremes being −10 and +15. The best medium for the isolation of the organism is litmus nutrose agar made with meat infusion (Eyre). The best medium for continuous cultivation is beef serum agar, reaction +8.

*Plain Agar Plates.*—Colonies grow slowly and reamin small. At the end of four to five days they are about ½ mm. in diameter, pale and delicate. Under low power they are round, regular, uniform, translucent, with entire margins. Deep colonies pin-point. Under low power biconvex disks. In eight to ten days the surface colonies are raised, whitish, somewhat larger, ½ to 1 mm. in size.

*Plain Agar Slant.*—Growth slow, barely visible in twenty-four hours. In forty-eight hours a faint, film appears along the line of inoculation. With moist agar this spreads slightly but remains thin and delicate. On dry agar the growth may become granular.

*Plain Agar Stab.*—Delicate growth along the line of inoculation in two to three days with a slight surface expansion. Line growth somewhat more abundant in eight to nine days, surface growth still delicate.

*Dextrose Agar Plates.*—Growth slow, surface colonies appearing in four to five days, round and circumscribed, ½ to 1 mm. in diameter. Under low power round and regular, somewhat thicker in the center, margins thin, edges entire. Deep colonies pin-point. Under low power biconvex disks. After ten to twelve days the colonies are slightly larger. No acid.

*Dextrose Agar Slant.*—Delicate, flat, non-spreading growth along the line of inoculation in forty-eight hours. Somewhat thicker after four to five days. No acid.

*Glycerin Agar.*—Growth like that on ordinary agar.

*Gelatin Plates.*—Small delicate colonies after four to five days at 22° C. No liquefaction.

*Gelatin Stab.*—Delicate line growth in four to five days. No liquefaction. White flocculent precipitate in 10 per cent. fluid gelatin at 37° C. (Eyre). Gelatin usually clear above, but may be turbid.

*Fluid Blood-serum.*—Fine flocculent precipitate, fluid above clear, but may be turbid.

*Loeffler's Blood-serum.*—Faint, delicate, rather dry growth in three to four days. Slight increase after eight to ten days.

*Broth and Peptone.*—Faint turbidity and scanty sediment in three to four days. No superficial film.

*Litmus Milk.*—Rapid development of an alkaline reaction, milk light blue in color. Reaction better seen in goat's milk than in cow's milk. Alkaline reaction in *neutral litmus milk-whey*, after eight days corresponding to 0.2 to 0.3 per cent. N/10 $H_2SO_4$.

*Potato.*—Invisible growth on acid potato. On alkaline potato moist, white to yellow growth after four to five days.

*Dextrose Fermentation Tubes.*—Growth limited to open bulb. Arm clear. No acid. Carbohydrates not fermented.

**Resistance.**—Thermal death point 57.5° C. in ten minutes. Destroyed by drying on coverslips at 90° to 95° C. Destroyed by direct sunlight in a few minutes to one and a half hours (Horrocks[4]). Resistant to diffuse daylight seven days (Zammit[5]). Alive after eighty-three days in dry sterilized earth containing dung. May live on moist coverslips and filter paper twenty-one days, on wool ninety days. On agar cultures kept from drying, alive after eight hundred and thirty days at 18° to 22° C. Ordinarily lives two hundred and ninety-six to three hundred and ninety days on agar. Alive in litmus milk after two hundred days, in broth after one hundred and seventy-three days (Shaw). Dies rapidly in sterile water, alive after five to seven weeks in sterilized tap water and sea-water. Not isolated after fourteen to twenty-one days from non-sterilized tap water and sea-water. Destroyed by 1 per cent. phenol in two and one-half to fifteen minutes, by 0.5 per cent. phenol in one hour, by 5 per cent. bichlorid of mercury in two and one-half to fifteen minutes.

**Pathogenic Action.**—When first isolated virulent to guinea-pigs and rabbits by subcutaneous, intravenous, and intraperitoneal inoculation. The animals live a long time, one-hundred to two hundred days. After repeated passage through animals the strains become more virulent, killing in twenty-four to seventy-two hours by intravenous inoculation, three to twelve days by intraperitoneal and nine to thirty-six days by intracranial injection (Carbone, Eyre). In death from acute infection the organisms are present in all the organs and tissues. With male animals a double purulent vaginalitis may occur with a beginning hypertrophy of the testicle, the pus containing many organisms. In chronic infection the organisms may be isolated from the kidneys and the urine, in small numbers from the spleen and bone-marrow, but not from the liver. The spleen and bone-marrow are often sterile however.

With rats and mice the organisms are pathogenic at first only by intracranial injection. After several cerebral passages the strains have an increased virulence and are pathogenic by all routes. On section the glands are enlarged and the spleen hypertrophied. Pathogenic also to dogs, horses, mules, cows, sheep, goats, and apes. In apes, *Macacus rhesus* and Bonnet-apes, intravenous and subcutaneous injections produce a fever like Malta fever in man. Spontaneous infection in many animals, horses, mules, cows, sheep, and goats. Goats are intensely infected on the Island of Malta. The Mediterranean Fever Commission in 1905 and 1906

found 30 per cent. of the goats on the island infected, with 10 per cent. carriers with organisms in their milk.

Agglutinins for *Bacillus melitensis* found in spontaneously infected animals, (40 per cent. of the goats on the island giving an agglutination). They are found also in the serum of immunized rabbits and guinea-pigs. Not in rats and mice.

In *man* the diagnosis can be established by agglutination which is positive after the fifth day (Aldridge, Basset-Smith,[6] Gilmour), rarely on the first day, or later. Agglutinins are present in large quantity, up to 1 : 1000 or 1 : 5000 during the first few days. They may run up to 1 : 500,000 (Eyre). Normal serum agglutinates the organism at 1 to 10 (Birt,[7] Lamb), partially at 1 to 20. *Bacillus melitensis* can also be obtained by splenic puncture and from the blood during life. Blood cultures are positive from the second day and up to three hundred days. They are found in the majority of the cases, 68 per cent. in (one hundred and three examinations (Shaw); 82 per cent. in 45 cases (Gilmour). *Bacillus melitensis* is also present in the urine in many patients after the fifteenth day.

Laboratory infections with this organism are not uncommon (Koose[8]) and may result fatally.

1. Bruce: J. Roy. Army Med. Corps, 1907, viii, 225.
———: Tr. Epidemiol. Soc., 1907.
2. Hughes: Brit. M. J., 1899, ii, 657.
3. Eyre: Mittelmeerfieber. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 1912, iv, 421.
4. Horrocks: J. Roy. Army Med. Corps, 1907, i, 426.
5. Zammit: J. Roy. Army Med. Corps, 1908, x, 219.
6. Basset-Smith: J. Trop. M. and Hyg., 1907, x, 169; 170.
7. Birt: Brit. M. J., 1907, ii, 1336.
8. Koose: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1924, xci, 493.

Fig. 97.—*Bacterium abortum* from fourteen-day culture plain agar stab, stained with carbol-fuchsin.

### Bacterium abortum Bang

Discovered by Bang[1] in infectious abortion of cattle and now established as the principal agent of the disease. Widely distributed in cattle in America.

**Morphology.**—The organisms are present in the infected tissues in great numbers, either as single individuals or in large bunches of closely approximated bacteria, frequently inside the cells. They are very small, appearing either as cocci or short oval bacilli. Under dark-field illumination one or two round glistening bodies are seen inside the rods and the two ends appear darker. In old broth cultures involution forms develop. They are long, thick, irregularly-stained rods with swollen ends, distended ovoid structures, and coccoid bodies. Length 1 to 2 $\mu$. Width 0.3 to 0.8 $\mu$.

**Staining Reactions.**—When stained the organisms resemble fowl-cholera or swine-plague. They stain with the usual anilin dyes, best with carbol-thionin, methylene-blue or borax methylene-blue, and especially well with Leishman's and Giemsa's stains. Non-motile.

**Cultivation.**—In Bang's early experience it was found that the abortion bacillus was hard to cultivate in the first few generations, but after once accustomed to artificial conditions could be transferred without difficulty. It does not ordiarily grow when the atmospheric oxygen is removed by pyrogallic acid solution. According to Stribolt growth occurs on the surface of media when the atmospheric air is replaced by pure oxygen, the organisms notwithstanding the concentration of oxygen in the atmosphere, 21 per cent., while a concentrated oxygen favors their development. Bang described two oxygen optima for *Bacillus abortus*, one when the oxygen tension is less than that of the atmospheric oxygen, the other when it is slightly under 100 per cent. Between these two zones the organisms develop badly or not at all. This zone growth is shown in serum-agar stabs in an atmosphere of pure oxygen, one zone of colonies occurring just below the surface and another just above the bottom. Since Bang's pioneer work a number of media have been devised for this organism, and it has been found that surface growth may occur from the start provided the right medium be employed.

Cultures may be obtained by a variety of methods. According to Bang and Stribolt excellent results are obtained with a medium containing $\frac{3}{4}$ per cent. of agar and 5 per cent. of gelatin in meat-infusion peptone, cooled to 45° C. and mixed with half the quantity of sterile fluid serum. Shake cultures are made in the usual way. About 0.5 cm. below the surface a zone of growth develops, 1 to 1.5 cm. deep, made up of very small punctiform pin-head colonies with slightly jagged edges. The lower limit of growth is in just about the same situation as the upper limit of growth of a strict anaërobe. Later the colonies become larger, the size of pepper grains, with very jagged edges, brownish in color. Nowak[2] found that *Bacillus abortus* grows well in sealed tubes in which the oxygen is removed by the growth of *Bacillus subtilis*. By gradually diminishing the amount of the "subtilis" culture *Bacillus abortus* was slowly accustomed to aërobic conditions. Smith and Fabyan[3] have successfully employed *Bacillus coli* and *Bacillus megatherium* for the production of the same type of symbiotic growth. Macfadyen and Stockman[4] report that *Bacillus abortus* will also grow in serum-gelatin-agar in a grayish cloudy ring immediately under the surface of the medium or as many small isolated flecks along the glass wall at the surface. Frequently the first sign of growth is in the water of condensation. Sometimes the organisms, when first isolated, and frequently after cultivation in the laboratory, grow near the surface of the media as round, sharply circumscribed, grayish-white or bluish transparent colonies, about the size of millet seeds or heads of pins (Zwick and Wedeman). These colonies coalesce until eventually the surface of the medium is covered by a homogeneous grayish-white or grayish-yellow expansion. Occasionally the abortion bacillus can be cultivated from infected material directly on ordinary slant serum agar under *aërobic* conditions. To avoid the use of serum which clots on heating the serum may be diluted two to three times with distilled water, neutralized or made slightly alkaline. *Agar gelatin* also serves for the cultivation of the abortion bacillus, when the tubes are sealed with paraffin. The first generations are scanty, but later growth abundant.

*Agar Plates.*—Small bluish, shimmering, punctiform colonies. The superficial develop and resemble the agar gelatin colonies in size and form, while the deep remain opaque, round or whetstone in shape, and dark brownish-yellow in color. Edges of the superficial and deep colonies smooth, seldom irregular.

*Agar Slant.*—Growth either as isolated punctiform colonies like those on agar gelatin, or as a delicate dew-like expansion which gradually take on a grayish-blue, grayish-yellow, or brown color. At the bottom of the tube a grayish-white or grayish-yellow flocculent sediment develops in the water of condensation which remains clear above it. It has recently been shown by Smith[5] that good agar surface growth is brought out in early generations by the introduction of $CO_2$, $\frac{1}{4}$ to 10 per cent., and even down to 0.1 per cent.

*Agar Stab.*—Growth in the neighborhood of the point of puncture as a grayish-blue expansion, which spreads and eventually covers the entire surface of the medium. Along the line of inoculation the growth develops after six to eight days either as isolated colonies or as a narrow grayish-yellow streak. The growth may occur to a depth of 2 cm. or in old cultures along the entire line.

*Gelatin Plates.*—Colonies develop very slowly, usually after weeks. They are like those on agar plates.

*Gelatin Slant.*—After eight to fourteen days a thin grayish-white expansion with a bluish shimmer by transmitted light.

*Gelatin Stab.*—Delicate growth at point of puncture as a grayish-white expansion which

has about the diameter of a pepper grain after two to three weeks. About this time growth occurs along the line of inoculation as a narrow grayish-white or yellowish-white streak. Later this is yellow-brown.

*Gelatin Shake Cultures.*—Grayish, uniformly turbid zone just below the surface and extending to a depth of ½ to 1 cm. No growth on the surface. In older cultures individual colonies appear in this cloud-like zone, at first grayish-white, later brownish-yellow. Gelatin not liquefied.

*Potato.*—Rather abundant development beginning after eight to fourteen days, either as a grayish-white to grayish-brown glistening mass or as a very thin, gray, dull glistening expansion. The character of the growth seems to vary with the composition of the potato.

*Blood-serum.*—Growth like that on agar, but less abundant.

*Glycerin broth* (5 per cent.) containing sterile serum in proportion of two to one shows a very sparse fine sediment after fourteen days. This sediment is made up of small white granules, colonies of the organism. If oxygen be passed through the medium and the tubes closed with paraffin a diffuse turbidity and an abundant fine sediment are produced. In *horse* or *beef serum* diluted with twice the quantity of distilled water, made alkaline and sterilized, then added to equal parts of 6 per cent. glycerin broth, and finally receiving 1 per cent. glucose, an abundant development occurs after two to three weeks. The serum can be replaced by amniotic fluid. In this latter medium a slight turbidity develops with a whitish, crumbly, flocculent sediment, a similar flocculent growth occurs on the surface along the glass wall, and frequently the entire surface is covered with a thick film from which tuft-like processes extend in the depths. Little growth in ordinary broth with or without glucose.

Some turbidity and flocculent sediment in *amniotic fluid.* Slight growth in *beef serum* as a grayish-white crumbly sediment. Little growth in *peptone solution*, slight turbidity, and grayish-white sediment.

Good growth in *milk* without change in reaction. In *litmus whey* a slight diffuse turbidity develops with a pale blue color, which occasionally changes to reddish and rarely goes back to the original color.

*Special Media.*—Grayish-white or grayish-blue expansion on v. Drigalski-Conradi medium without changing its color. Sparse gray-white growth on Endo's medium, becoming more abundant and light rose-red. The medium also becomes light rose-red. Moderate growth on malachite green agar and on Conradi's brilliant green picric acid agar, grayish-yellow to yellowish-green in color.

*Bacillus abortus* produces reducing substances which act on litmus. No indol, $H_2S$ positive. No acid or gas in carbohydrates. Evans[6] states that the organism commonly decomposes nitrates, asparagin, and urea, and turns milk alkaline.

**Occurrence and Survival.**—Found in aborting cattle in the uterine exudate and in the aborted fetus in the stomach and small intestine, in the blood, in the medulla oblongata, and in all the organs. Found by Stribold in the mummified fetus in an animal whose conception started at least nine months earlier. The organisms seem able to survive a long time in the tissues. Bang has shown that they can survive five to nine months in the uterus. Outside the body they survive also a long period. Macfadyen and Stockman found them alive and virulent in the uterine exudate preserved in broth seven months. They die out quickly in cow urine and in sterilized dry cow-dung, but survive long periods in sterile moist cow-dung.

*Destroyed* in two hours at 55° C. (Macfadyen and Stockman), at 60° C. in ten to fifteen minutes, at 65° C. in five to ten minutes (Zwick and Wedeman[7]).

**Pathogenic Action.**—Pathogenic to mice, rats, and guinea-pigs. In mice ¼ to ½ c.c. serum-broth culture produces fatal infection in two to four days when injected intraperitoneally. At autopsy the organisms are present abundantly in the peritoneal cavity, but in the blood only in small numbers. Subcutaneous injection is followed by a deep local necrosis. Similar effects in rats from larger quantities ½ to 1 c.c. In guinea-pigs Smith and Fabyan have shown that the lesions resemble tuberculosis. The spleen is swollen and yellowish nodules are found in the liver. The kidneys are enlarged and small gray foci occur in them, while in the lungs grayish tubercle-like nodules appear. Large doses are required to bring on infection and the animals die three to four months after inoculation, either subcutaneous or intraperitoneal. Rabbits are usually resistant even to large doses. In large animals (cows) Bang has produced abortion by the direct injection of abortion bacilli into the vagina and by feeding material containing the organisms. He also has produced it by intravaginal and intravenous administration in sheep, goats, mares, and rabbits. Macfadyen and Stockman have produced abortion in cows by intravaginal, subcutaneous, and intravenous administration. Nowak also produced abortion in pregnant rabbits and guinea-pigs by the subcutaneous, intraperitoneal, and intravenous route. In the natural disease agglutinins and complement-fixing substances appear in the blood-stream. A preparation named "abortin" has been made from cultures by Macfadyen and Stockman for diagnostic purposes, but has not come into general use. Relation of *Bacterium abortum* and *Bacterium melitense.* Evans regards the abortion bacillus of Bang as practically identical with the organisms which cause Malta Fever. There is no difference in cultural reaction, and reciprocal agglutinations can be demonstrated in immunized

animals. Meyer[7] and Shaw[8] have pointed out that the pathogenic action on apes is not quite the same in the two organisms. Thus the abortion bacillus has but little invasive power for these animals, while *Bacterium melitense* is highly virulent for them, small quantities producing a general infection. The two strains apparently represent the same original stock, the abortion bacillus being accustomed to a parasitic life in cattle, the organism and Malta Fever being accustomed to a similar life in goats and man. Infection of man with Bang's bacillus has not yet been demonstrated with certainty. Meyer and Shaw[8] suggest that the two organisms should be placed together in a new genus, *Brucella.*

1. Bang: Deutsche Ztschr. f. Thiermed., 1897, i, 241. Arch. f. wissensch. u. prakt. Tierh., 1903, xxxiii, 312.
2. Nowak: Ann. de l'Inst. Pasteur., 1908, xxii, 541.
3. Smith and Fabyan: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1911, lxi, Orig., 549.
4. Macfadyen and Stockman: Rep. of the Dept. Com. to inquire into epizoötic abortion. London, 1909.
5. Smith: J. Exper. M., 1924, xl, 219.
6. Evans: J. Infect. Dis., 1916, xviii, 437.
7. Zwick and Wedeman: Arb. a. d. K. Gsndhtsamte, 1912, xliii, H. 1.
8. Meyer and Shaw: J. Infect. Dis., 1920, xxvii, 174.

### Bacillus abortus Variety lipolyticus Evans

Isolated by Evans from milk and characterized by its ability to hydrolize butter fat. Slender rods 0.8 to 1.5 $\mu$ in length. On meat infusion agar slants delicate growth as very small separate colonies, scattered over entire surface of medium if this be moist, or a ribbon-like growth along the line of inoculation if this be dry. A good medium is agar containing 5 per cent. bile. The best medium is agar containing 10 per cent. blood-serum. Here the growth is vigorous and fairly rapid. In a few days the medium takes on a brownish tinge which deepens with the age of the culture. When agar shake cultures are heavily seeded throughout the entire mass growth takes place in a thin layer a few millimeters below the surface just as in *Bacillus abortus* cultures. No effect on gelatin, nitrates, urea, or asparagin. No acid from carbohydrates and related substances. Little growth in litmus milk from which the cream has been removed. In whole litmus milk a rapid multiplication and slow development of acid occurs and is evident at first in the cream layer. After two weeks at 37° C., the optimum temperature, the cream layer is converted into a firm, slightly granular, ill-smelling mass.

### Bacillus abortus Variety B

Isolated by Evans from milk. Morphology similar to that of *Bacillus abortus.* Slender rods 0.8 to 1.5 in length. Acid from dextrose and maltose. No action on butter fat. Growth abundant on infusion agar, dirty yellowish in color. In agar shake cultures colonies grew on the surface, and in the tupper 6 to 10 millimeters of agar. Asparagin, nitrates, and urea decomposed. No liquefaction of gelatin.

### Bacillus abortus Variety C

Renders broth media slightly alkaline and occasionally liquefies gelatin. Slender rods 0.8 to 1.5 $\mu$ in length.

Evans: J. Infect. Dis., 1916, xviii, 437.

### Bacillus bronchisepticus Ferry

Obtained by Ferry[1] from cases of distemper in dogs and possibly identical with the organism of dog's distemper found the same year by M'Gowan.[2] The findings of Ferry and McGowan were subsequently confirmed by Torrey and Rahe,[3] who distinguished two types, A and B.

**Morphology.**—Small bacilli about the size of the organisms of the hemorrhagic septicemia group. On plain agar in twenty-four hours they measure 0.3 to 0.4 $\mu$ in breadth by 0.7 to 1 $\mu$ in length. No increase in size on dextrose agar. No spores. According to Torrey and Rahe Type A is longer than Type B, measuring 0.4 to 0.5 $\mu$ in breadth by 1 to 1.6 $\mu$ in length, Type B measuring 0.4 to 0.5 $\mu$ in breadth by 0.75 to 1.25 $\mu$ in length. In fluid media the organisms stain more deeply and chains and threads of 50 or more appear. In five days swollen and involution forms are found. Type A measuring 0.6 to 0.75 $\mu$ in breadth by 1.5 to 2 $\mu$ in length, Type B measuring 0.6 $\mu$ by 1.5 to 1.6 $\mu$.

**Motility** active. Flagella 5 to 6 in number several times the body of the bacillus in length (Torrey and Rahe).

**Stains** with ordinary anilin dyes, but somewhat less readily than most bacteria. Gram-negative. Organisms often show bipolar staining especially from glycerin agar.

**Cultivated** from cases of distemper best from the smaller bronchi and trachea where it is often in pure culture. Grows better with the free access of oxygen than without it. Optimum temperature 37° C. Grows at 20° to 40° C. Range of reaction −3 to +3 to phenolphthalein.

*Plain Agar Plates.*—Colonies in twenty-four hours exceedingly small, pin-point, translucent, slightly raised. In forty-eight hours the surface colonies are slightly larger, ½ to 1 mm. in size, round, convex, smooth, amorphous, and translucent. Under low power they are slightly granular, with dark centers, lighter peripheries, edges entire. Deep colonies pin-point, under low power biconvex disks. The colonies grow slowly and at the end of six to seven days are larger, 2 to 3 mm. in diameter, thicker, with a grumose center and undulate edges. When separated the colonies may be somewhat larger.

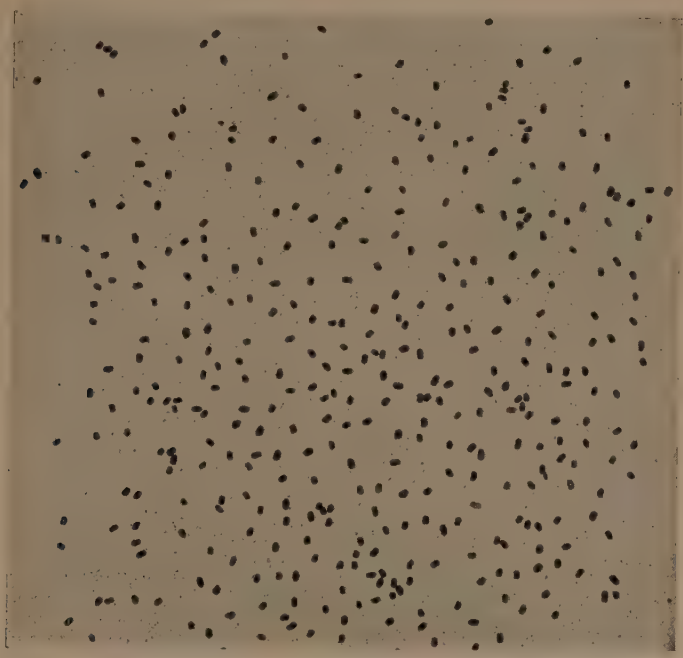

Fig. 98.—*Bacillus bronchisepticus* from plain agar twenty-four hours old, stained with gentian-violet.

*Plain Agar Slant.*—Delicate non-spreading growth in twenty-four hours, fairly abundant in two to three days, thicker at the bottom. In seven to eight days the growth is moderately thick, dull white.

*Plain Agar Stab.*—Delicate line and surface growth in twenty-four hours, somewhat increased in forty-eight hours. In seven to eight days the line growth is more marked and the surface growth thick and spreading.

*Dextrose Agar Plates.*—Colonies appear on the surface in twenty-four to forty-eight hours, round, regular, opaque, ¼ to ½ mm. in size. Under low power they are faintly granular, translucent, darker in the center, lighter at the periphery, with edges entire. Deep colonies pin-point, under low power biconvex disks. After three to four days the colonies are larger with dense centers and lighter, hazy peripheries. Under low power the edges are broken and show a slight tendency to spread. After eight to ten days the colonies may show concentric rings of growth. No acid, plates turning dark blue.

*Dextrose Agar Slant.*—Moist whitish growth in twenty-four to forty-eight hours. After five to six days the growth increases in intensity and the agar becomes alkaline and dark blue in color.

*Gelatin colonies* grow very slowly at 22° C., appearing small and fine after five to six days. Under low power round, regular, and uniform. After ten days the colonies are still small and fine. No liquefaction.

*Gelatin Stab.*—Delicate line and surface growth in twenty-four to forty-eight hours. After four to five days the growth is thicker in the depths and spreads slightly on the surface. In older cultures, ten to twelve days, the surface growth is quite abundant, the line growth still delicate.

*Blood-serum.*—Thin delicate growth in twenty-four to forty-eight hours, more abundant in four to five days.

*Potato.*—Rather thick raised growth in twenty-four hours, sticky, light tan in color, emiting a decidedly stale odor. After four to five days the growth is thicker and darker, shiny, often yellowish-brown, with a brownish discoloration of the potato. In old cultures the growth may be brownish-red.

*Plain milk* becomes translucent in four to five days. After seven days the translucence is marked. Later, fifteen to twenty days, the medium is changed to a clear yellow watery fluid with a slight sediment (Torrey and Rahe).

*Litmus Milk.*—An alkaline reaction begins in two days. After seventy-two hours the upper half of the tube is dark blue and in five days the milk is dark blue throughout. In old milk cultures the litmus may be decolorized at the bottom.

*Broth.*—Turbidity in twenty-four hours, increased in forty-eight hours, without surface film. In older cultures the turbidity is increased still more and a faint sediment forms. The broth does not clear.

*Peptone.*—Growth like that in broth, turbidity and faint sediment without scum.

*Dextrose Fermentation Tube.*—Turbidity in the bowl, closed arm clear. No acid. No fermentation of dextrose, mannite, maltose, lactose, or saccharose.

Indol not produced. Nitrates not acted on by Type A, reduced to nitrites by Type B. Hemolysins produced for dogs, rabbits, and guinea-pig erythrocytes, demonstrable on blood agar plates (Torrey and Rahe).

Destroyed in broth and saline, in ten minutes at 55° C. (Type A) or at twenty minutes at 55° in broth, twenty-three minutes in saline (Type B).

Destroyed in thirty-five to forty-five minutes in dry heat at 75°, in less than five minutes at 100° C.

Resists drying in dark up to five months. Dead in six months. Viable after exposure twenty-nine days to winter temperature, 0° C. to −19.5° C. Dead after forty-three days. Survives ten minutes exposure to sunlight, not 15 (Type A), two minutes not 5 (Type B).

Destroyed in 1 per cent. lysol at once, in 0.5 per cent. in ten minutes, 0.1 per cent. having no effect in an hour. Killed by 1 per cent. phenol in less than five minutes, by 0.5 per cent. in fifteen minutes, Killed in less than five minutes by 30 to 100 per cent. alcohol, 10 per cent. having no effect in an hour. Destroyed by bichlorid of mercury, 1 : 100,000, in less than five minutes, by 0.5 per cent. formaldehyd in ten minutes or less, by 0.1 per cent. in thirty to thirty-five minutes.

**Pathogenic Action.**—With *Bacillus bronchisepticus* Ferry[4] has produced distemper in puppies by injection into the trachea, the animals showing cough, nasal catarrh, conjunctivitis, bronchopneumonia, diarrhea, and a pustular eruption on the skin. Distemper was also produced in puppies by Torrey and Rahe, by intranasal and intraoral administration as well as by intratracheal injection. The organisms are rarely present in the blood. Recovered dogs are immune to the organism and also to the natural distemper. Agglutinins are present in the blood-stream of immunized dogs and also in dogs suffering from natural distemper. Highly toxic to guinea-pigs, ½ mg. of a twenty-four-hour glycerin agar culture killing animals of 200 gram weight in one to four days. Sterile filtrates are also highly toxic to guinea-pigs. Immunized animals show an agglutination for *Bacillus bronchisepticus* in a dilution of 1 : 4000, but no antitoxic action. Ferry[5] has also reported an epidemic from *Bacillus bronchisepticus* in guinea-pigs, rabbits, and monkeys.

1. Ferry: Am. Vet. Rev., 1910, p. 499.
2. M'Gowan: J. Path. and Bacteriol., 1911, xv, 372.
3. Torrey and Rahe: J. Med. Research, 1912–13, xxvii (n. s. xxii), 291.
4. Ferry: J. Infect. Dis., 1911, viii, 399.
5. Ferry: Am. Vet. Rev., 1912.
———: Vet. J., 1912, lxviii.

### M'GOWAN'S ORGANISM OF DOG DISTEMPER

Isolated by M'Gowan from a laboratory epidemic of distemper in dogs, ferrets, monkeys, goats, guinea-pigs, rabbits, cats, and in one human case from the nasal secretions. The animals showed symptoms generally regarded as pathognomonic of distemper, *i. e.*, purulent discharge from eyes, with corneal ulcers, discharge from nose, sneezing, coughing, fever, diarrhea, anorexia, emaciation, paralysis, chorea, etc. The organism is regarded by

M'Gowan as different from the members of the hemorrhagic septicemia group.

**Morphology.**—Short bacillus with faint polar staining, 0.4 to 0.5 $\mu$ in width by 0.5 to 2.3 $\mu$ in length. Arranged in chains and in small groups in broth and in tissues. No spores.

**Stains** with anilin dyes, carbol-thionin showing the polar granules. Gram-negative, decolorizing easily.

*Grows* readily on all the usual culture media.

**Cultural Characters.**—*Agar Slant.*—In twenty-four hours a uniform, thin, gray, moist, slightly tenacious growth from heavy seeding. With light seeding the colonies appear as discrete dew points. These grow rapidly in twenty-four hours, becoming as large as pin-heads. They are raised above the surface, regular hemispheres in shape, and have an opaque white porcelanous look with an opalescent sheen. They look as if little pearls had been half-buried in the agar. They grow rapidly, losing their hemispherical shape and spreading slightly.

*Blood Agar.*—Growth more rapid than on ordinary agar with the same characteristics, except that the white color of the colonies is accentuated.

*Glycerin Agar.*—Good growth.

Turbidity in *broth, glucose broth,* and *peptone water.* The liquid remains turbid and a precipitate settles to the bottom. No pellicle.

No acid or gas in lactose, saccharose, salicin, mannite, dulcite, maltose, galactose, raffinose, glucose, inulin, inosite, and adonite. The media become alkaline.

*Litmus Milk.*—Alkalinity, beginning in twenty-four hours and increasing. No coagulation.

*Potato.*—Characteristic growth, buff-colored yellow to copper-brown, raised, moist, heavy, appearing in twenty-four to forty-eight hours. Growth the same on normal acid, or neutralized potato.

*Gelatin.*—Growth slow with tendency to the formation of discrete colonies. No liquefaction. No gas bubbles. In stab cultures a funnel-shaped cupping due to desiccation.

Good growth on *blood-serum.*

Good anaërobic growth in the depth of stab cultures in glucose agar and in Buchner's tubes with pyrogallate of soda.

No indol.

No reduction of neutral red in lactose neutral red peptone solution. Alkali formed.

*Killed* in half an hour by exposure to 50° C.

Resistant to cold, exposure to a temperature of −6° C. to 21.5° C. not destroying it in four days. Not destroyed by exposure to strong sunlight for three days.

**Pathogenic Action.**—Subcutaneous inoculations virulent to mice.

Intraperitoneal inoculations virulent to puppies, cats, guinea-pigs, rats, mice, and pigeons. A disease resembling distemper can be produced in puppies by introduction into the nose. Agglutinins produced against the organism by immunization, 1 to 60 or 1 to 120. Animals dead of infection do not show hemorrhages or septicemia.

M'Gowan: J. Path. and Bacteriol., 1911, xv, 372.

### Tartakowsky's Bacillus of Guinea-pig Pneumonia

Originally described by Tartakowsky.[1] Found in this country by Theobold Smith,[2] who regards it as identical with *Bacterium pneumoniæ caviarum* of Strada and Traina,[3] *Bacterium pulmonum glutinosus* of *Martini*,[4] and *Bacillus caviseptícus mobilis* of Selter.[5] Possibly the same as M'Gowan's organism of dog distemper and possibly identical with the organism described by Mallory in the ciliated epithelium of the bronchi in animals treated with pertussis vaccine.

**Morphology.**—Small bacillus with rounded ends, measuring about 0.5 $\mu$ in width by 0.7 $\mu$ in length with occasional long rods. Motility active. Flagella up to 6 in number, shorter with somewhat thicker undulations of lower amplitude than the flagella of *Bacillus typhosus.*

*Stains* easily by the ordinary anilin dyes. Gram-negative.

**Cultivated** easily on *gelatin* under aërobic conditions. Surface colonies appear as disks 2 to 5–6 mm. in diameter. The larger colonies have irregularly lobed borders, thick centers, with ridges radiating to a marginal zone which is very delicate and transparent. They slightly suggest the gelatin colonies of *Bacillus coli.* The smaller colonies are somewhat more compact, lacking the marginal zones. Deep colonies in thirty-six to forty-eight hours are minute spheres or homogeneous disks which later show one or more concentric marginal rings. They are not over 0.5 $\mu$ in diameter. No coarse granulations.

*Loeffler's Serum.*—Fairly rich pale yellow layer in one to two days.

*Potato.*—Rich yellow-brown growth in six to seven days, exactly like that of *Bacillus mallei* or like that of *Bacillus abortus*.

*Broth.*—Very delicate surface membrane like tissue paper in forty-eight hours. Broth very ropy and turbid. Distinctly viscid in cultures several weeks old on boiling. The addition of a few drops of acetic acid induces a separation of this viscid material as twisted strands or minute flakes. It is apparently a substance like mucin.

No change in milk.

No indol.

No acid or gas with sugars.

*Pathogenic* to guinea-pigs, subcutaneous inoculation giving rise to a large painful swelling which may ulcerate. Intra-abdominal injection produces hemorrhages with a viscid translucent exudate and pseudomembranes on the liver and spleen and the less mobile parts of the intestine. Intrathoracic inoculation gives rise to a pleural exudate.

*Filtered* through Berkefeld No. 5 by Smith and by Wherry.[6]

1. Tartakowsky: Arch. d. sc. biol., 1898, vi.
2. Smith: J. Med. Research, 1913–14, xxix (n. s. xxiv), 291.
3. Strada and Traina: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1900, xxviii, 635.
4. Martini: Arch. f. Hyg., 1900, xxxviii, 114.
5. Selter: Ztschr. f. Hyg., 1906, xliv, 346.
6. Wherry: J. Med. Research, 1902, viii (new series, iii), 322.

### Bacterium Group VII

Encapsulated non-motile, non-liquefying organisms, usually pathogenic to man and to small animals. Producing acid and gas or acid alone from dextrose, saccharose, or lactose.

### Bacillus Mucosus Capsulatus Group

A large number of encapsulated Gram-negative bacilli with marked pathogenic properties have been isolated from man and animals and have received a variety of names. The first of these to be definitely recognized was *Bacterium lactis aërogenes*, found in the intestinal tract of young children by Escherich[1] and at that time regarded as closely related to *Bacillus coli communis*. Subsequently an encapsulated organism, *Bacterium pneumoniæ* was isolated by Friedländer[2] from cases of croupous and bronchopneumonia and thought to be the principle etiological agent in pneumonia. Encapsulated organisms like Friedländer's bacillus were soon found in many other lesions, and usually called *Bacillus mucosus capsulatus* or put together in a group called the *Bacillus mucosus capsulatus* group. Some of these are *Bacillus mucosus capsulatus* of Fasching[3] and of Fricke, *Bacillus canalis capsulatus* of Mori,[4] *Bacillus capsulatus* of Pfeiffer,[5] *Bacillus icterogenes capsulatus* of Bandi, *Bacillus ozænæ* of Abel[6] and *Proteus hominis capsulatus* of Bordoni-Uffreduzzi. Representatives of this group have been extensively investigated in America by Howard[7] and Perkins[8] among others. Comparative studies of a great many strains revealed close similarity between the different members and the identity of many different strains. Some but not all of the encapsulated bacteria found by Escherich were recognized as members of this group, as well as some of the encapsulated bacteria found in milk and known as *Bacillus acidi lactici*. The morphological and cultural properties and the pathogenic action of the various strains are practically identical. Among early attempts to classify the members of the group are those of Wilde,[9] Strong,[10] and of Perkins. Perkins makes four main types of these encapsulated Gram-negative organisms.

1. *Bacterium lactis aërogenes* of Escherich.
   Ferments glucose, saccharose, and lactose.
2. *Bacterium acidi lactici* of Hueppe (*Bacterium duodenale*, Ford).
   Ferments glucose and lactose, but not saccharose.

3. *Bacterium pneumoniæ* of Friedländer.
   Ferments glucose and saccharose, but not lactose.
4. *Bacterium rhinoscleromatis* of v. Frisch.
   Produces acid, no gas, in various carbohydrates.

More exact methods of differentiation by chemical reaction indicate that this is a more homogeneous group than originally supposed. All these organisms have a considerable ability to overcome acid and produce an alkaline reaction as was originally shown by Durham for *Bacterium lactis aërogenes*. They are therefore methyl red negative. In addition they produce acetyl-methyl-carbinol and give a positive Voges-Proskauer reaction. On carbohydrate media they produce an original acidity, but quickly revert to alkali. The members of the group are thus easily differentiated from *Bacillus coli* or the various paratyphoid bacilli which are methyl red positive, Voges-Proskauer negative and permanently acid on sugar media. In addition the gases evolved from the carbohydrates are preponderantly carbon dioxid with a small amount of hydrogen. In the newer nomenclature of Clark and Lubs they are high ratio organisms as compared with the low ratio members of the "coli" group. There are three main types of organisms in the *Bacillus mucosus capsulatus* group, *Bacterium lactis aërogenes* of Escherich, *Bacterium pneumoniæ* of Friedländer, and *Bacterium rhinoscleromotis* of v. Frick. The organism known as *Bacillus acidi lactici* or *Bacterium duodenale* closely resembles *Bacterium lactis aërogenes*, differing only by its failure to ferment saccharose. Classification is based almost entirely upon cultural reactions, serological or immunological reactions not proving of great help in establishing relationships.

### BACTERIUM LACTIS AËROGENES Escherich

Described originally by Escherich in the dejecta of children fed on a milk diet and differentiated with some difficulty from *Bacillus coli communis*. Subsequently found to be encapsulated and to be related to the encapsulated organisms found vegetating on the nasal mucosa. Widely spread in the dejecta of man and animals.

**Morphology.**—Small rods with rounded ends measuring 0.5 to 0.6 $\mu$ in breadth by .75 to 1.25 $\mu$ in length. Long rods and shorter coccoid elements are common. Enclosed in a fairly large capsule which may embrace several individuals. No increase in size on dextrose agar. Non-motile. No flagella. No spores.

**Stained** easily by the ordinary anilin dyes, methylene-blue, gentian-violet or dilute carbolfuchsin. The capsular substance does not stain by these dyes but often forms a kind of débris about the organism. By gently washing the films in running water before staining more satisfactory results can be obtained. Gram-negative. The capsules may be demonstrated by the usual capsule stains, those of Welch, Hiss, Buerger, and Huntoon giving excellent results. They are most easily demonstrated in exudates in inoculated animals, but are found also in films from milk, and occasionally in freshly isolated cultures on plain agar and dextrose agar. In the intestines the organisms may show good capsules or at times the capsule may be difficult to demonstrate. The amount of capsular material present depends to some extent on the substance upon which the organism is growing. Fürst[11] believes that the large capsules occasionally seen consist of two ingredients, the true capsule belonging to the bacterial cell and not attacked by trypsin, and the so-called culture capsule about the bacterial cell, but belonging to the medium and soluble in trypsin. Hamm has found that the capsule consists of nucleo-albumin or nucleoprotein, not of mucin as previously taught.

**Cultivated** easily on the ordinary media. Growth most rapid at 37° C. slower at room temperature. Little growth below 12° C. Grows well on neutral, slightly alkaline, or slightly acid media.

*Plain Agar Plates.*—Colonies grow rapidly, appearing on the surface in twenty-four hours, raised, glairy, and viscid, 2 to 3 mm. in diameter. Under low power they are granular, often striated, frequently nucleated, edges abrupt, not spreading. Deep colonies well marked in twenty-four hours, under low power oval or disk-shaped. The surface colonies increase

Fig. 99.—*Bacterium lactis aërogenes* from plain agar, stained with gentian-violet, twenty-four hours old.

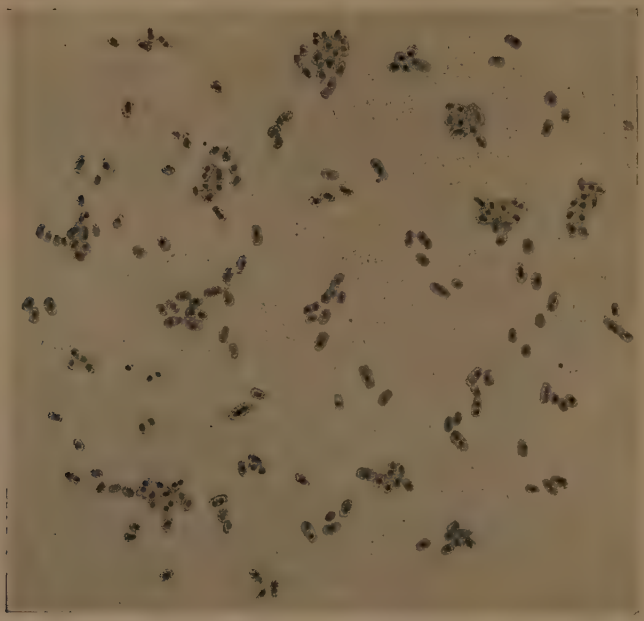

Fig. 100.—*Bacterium lactis aërogenes* from dextrose agar, stained for capsules.

rapidly in size and in four to five days are 3 to 4 mm. in size. When separated they may become quite large in ten to twelve days, 1 to 1½ cm. in diameter, with crenated margins. The deep colonies grow more slowly, but at the end of ten to twelve days may be distinct ovals and disks 1 mm. in diameter. Bottom colonies spread widely.

*Plain Agar Slant.*—Thick, moist, abundant, glairy, whitish growth in twenty-four hours. The growth increases rapidly in extent and spreads over the surface of the agar, especially near the lower part. The water of condensation may become quite turbid. In freshly isolated cultures the growth is viscid and long strings are brought away when touched with a platinum needle. The water of condensation may also be viscid.

*Plain Agar Stab.*—Abundant line and spreading surface growth in twenty-four hours. The growth increases rapidly forming a thick streak in the depths and a surface expansion which may reach the wall of the tube after eight to ten days. In old cultures the agar occasionally becomes brownish.

*Dextrose Agar Plates.*—Rapid growth, surface colonies appearing in twenty-four hours, raised, glairy, viscid, 2 to 4 mm. in size. Under low power dense and opaque, margins entire. Deep colonies large, ½ to 1 mm. in size, oval or biconvex disks; under low power dark and opaque. Agar acid with bubbles of gas. In two to three days the surface colonies are often 5 to 6 mm. in size, glistening, raised in the center, looking like large drops of dew. Later, eight to ten days, the surface colonies may be very large, 1 to 1½ cm. in size, thick, raised, and opaque, while the deep colonies are frequently large ovals or disks. Bottom colonies spread widely. The acid reaction gives way to an alkaline reaction which may appear in forty-eight hours on crowded plates later when the colonies are separated.

*Dextrose Agar Slant.*—Very abundant, elevated, moist, slightly yellowish growth in twenty-four hours; agar acid with bubbles of gas. In four to five days the growth is thicker and more abundant, the water of condensation turbid. The acid reaction gives way to an alkaline reaction in two to three days and in old cultures the agar is deep blue. Surface growth and water of condensation viscid.

*Gelatin Plates.*—Colonies appear in forty-eight hours, pale and delicate, 1½ to 2 mm. in diameter. Under low power they have darker centers with fringe-like peripheries. After four to five days the colonies may be much larger, 4 to 5 mm. in diameter, round or irregular with indented margins. Under low power these colonies may show distinctly petal-like peripheries. In older plates, ten to twelve days, the colonies have distinctly elevated central projections with irregular margins. No liquefaction.

*Gelatin Stab.*—Abundant line and moderate surface growth in twenty-four hours. The line growth becomes thicker in four to five days and the surface growth spreads from the point of puncture. In old cultures, ten to twelve days, the surface growth may reach the wall of the tube. No liquefaction. Gelatin may become brownish and show bundles of needle-like crystals.

*Gelatin Slant.*—White or grayish sperm-like expansion, often fluid and running down the gelatin so as to form an abundant viscid mass at the bottom.

*Blood-serum.*—Abundant, elevated, cream-colored growth in twenty-four hours, thicker and more wide-spread in two to three days. In old cultures the growth is very abundant and may be pale brown or cream-colored.

*Potato.*—Abundant, smooth, moist, yellowish growth in twenty-four hours. The growth increases rapidly in extent, becoming slightly yellow-brown. If the pieces of potato are immersed in water the potato starch is fermented and considerable gas is evolved.

*Litmus Milk.*—Rapid acidification and coagulation, usually in twenty-four to forty-eight hours. In four to five days the coagulum is hard and firm, usually white from a reduction of the litmus, with the expression of considerable yellowish whey. No liquefaction of the coagulum. Acetic, lactic, and succinic acid produced.

*Litmus Whey.*—Reddened and acid.

*Broth.*—Turbidity and abundant sediment without scum in twenty-four hours. Both turbidity and sediment become marked in four to five days. The broth does not clear, but may become viscid and sticky.

*Peptone.*—Turbidity and sediment in twenty-four hours, some ring growth. No scum. Later the turbidity becomes dense, the sediment heavy and a distinct film may form on the surface.

Good growth in *urine* as a diffuse turbidity and sediment. Reaction acid. Ammonia not formed.

Indol not produced. Methyl red negative. Voges-Proskauer positive.

*Dextrose Fermentation Tube.*—Turbidity and sediment in twenty-four hours, turbidity extending into the closed arm with an abundant evolution of gas which may collect in a column about 3 inches in height. Reaction acid. Over half the gas absorbed by KOH. Balance explosive. Gas formula: $\frac{H}{CO_2} = \frac{1}{1\text{–}1\frac{1}{2}}$. Saccharose and lactose fermented vigorously, in saccharose the gas frequently filling the closed arm. Gas formula the same.

*Resistance.*—*Bacillus lactis aërogenes* remains alive a long time in cultures and can frequently be transferred successfully after three months. Owing to its ability to overcome acid, the media becomes alkaline and the organisms survive. Destroyed slowly at 50° to 55° C. Killed at 60° C. in fifteen to twenty minutes. Somewhat resistant to light and to the action of disinfectants.

**Pathogenic** to rabbits, guinea-pigs, and mice. With subcutaneous injection, large doses,

2 to 3 c.c. of broth cultures twenty-four hours old, are required to kill. There is usually a small quantity of a glairy or whitish exudate at the site of inoculation consisting microscopically of fibrin, encapsulated bacilli and pus-cells. The adjacent lymph-glands are swollen. The organisms are distributed widely in the blood-stream and the animals present the picture of a septicemia. The spleen is engorged with blood, kidneys enlarged, liver shows cloudy swelling with small areas of necrosis. With intrapleural inoculation a pleurisy develops and occasionally a few areas of hepatization of the lung, the animals dying of a septicemia. After intraperitoneal inoculation a purulent peritonitis results and a generalized septicemia. Many cultures of *Bacillus lactis aërogenes* from the intestines are almost devoid of virulence.

*Varieties.*—In many instances strains of *Bacterium lactis aërogenes* do not present the typical picture described above. The organisms produce but little capsular substance and the surface cultures resemble those of *Bacillus coli*. They may be differentiated by their peculiar fermentation of sugars, their failure to give the methyl red test, their positive Voges-Proskauer and their ability to overcome acid. Old cultures of such strains on dextrose agar are alkaline, while old cultures of *Bacillus coli* are acid. Old laboratory strains and many intestinal strains resemble *Bacillus coli* at first. Such strains are apt to have a low virulence. After passage through animals they may become typical.

**Distribution.**—*Bacterium lactis aërogenes* is widely distributed in the intestines of man and animals. In children it is practically always present in the dejecta and normally is found in abundance in the upper part of the intestinal tract, less frequently in the lower portions. In adults it is also present in the feces and in the upper portions of the intestines. It is occasionally the cause of inflammatory conditions about the bowel and may be the etiological agent in cystitis, pyelonephritis, etc. It is constantly found in the dejecta of certain animals, especially in cattle. It invades the alimentary tract of calves within a few days after birth. It is practically always present in cow's milk and is some evidence of fecal contamination. It has not thus far been determined definitely whether the organisms described as "aërogenes" from the environment, and which as a rule are not encapsulated, represent this species or not.

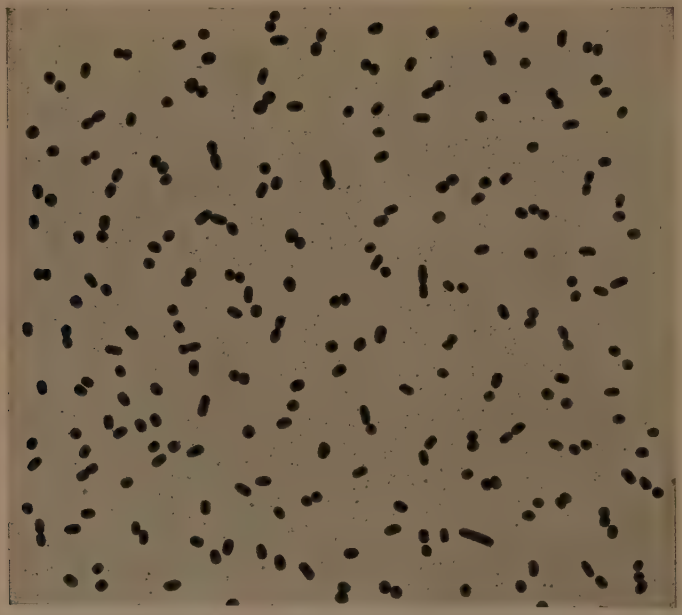

Fig. 101.—*Bacterium pneumoniæ* from plain agar twenty-four hours old, stained with gentian-violet.

### Bacillus acidi lactici Hueppe

Described originally by Hueppe.[12] Perkins has shown that this organism differs from *Bacillus lactis aërogenes* only by its failure to ferment saccharose. Spores were described by Hueppe in his original culture. If Hueppe's organism was a spore-bearing strain, the non-saccharose fermenting member

of this group should be known as *Bacterium duodenale.*[13] This member of the *Mucosus capsulatus* group is considerably rarer than either *Bacterium aërogenes* or *Bacterium pneumoniæ.* -

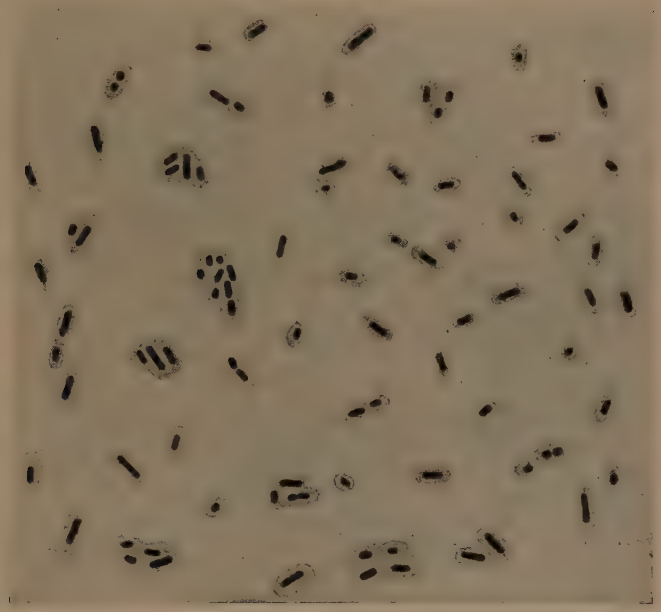

Fig. 102.—*Bacteria pneumoniæ* from plain agar twenty-four hours old, stained by Huntoon.

Fig. 103.—*Bacterium pneumoniæ* from dextrose agar twenty-four hours old, stained by Huntoon.

### BACTERIUM PNEUMONIÆ Freidländer

Discovered in the lungs in cases of lobar pneumonia in man by Friedländer. Subsequently shown to be the etiological agent in lobar pneumonia in a small number of cases and in many cases of bronchopneumonia.

Morphological and cultural characters like those of *Bacillus lactis aërogenes* as described above. The organisms are often somewhat longer, however, measuring 0.5 to 0.6 $\mu$ in breadth by 1 to 2 $\mu$ in length. The capsular substance is usually abundant, often enclosing several organisms. The bacteria are easily demonstrated by gentian-violet, methylene-blue, or carbolfuchsin, the capsule by Welch's, Hiss's, or Huntoon's stain. Gram-negative.

On *agar* the growth is very glairy, viscid, and stringy, the surface colonies large and the deep colonies well-developed spheres and disks. On *dextrose agar* the colonies grow rapidly, producing an acid reaction in the medium which soon gives way to alkali. Characteristic growth in *gelatin stabs* and in *plates* without liquefaction. Turbidity and sediment in *broth* and *peptone* without film.

*Milk* acid in twenty-four to forty-eight hours. Acidity permanent, not enough to coagulate the milk and not giving way to alkali. Methyl red reaction negative. Voges-Proskauer positive. No indol.

Ferments dextrose and saccharose actively, gas almost filling the closed arm. The bulk of this gas is absorbed by KOH, the balance explosive.

Gas formula $\frac{H}{CO_2} = \frac{1}{1-1\frac{1}{2}}$.

**Pathogenic Action.**—The Friedländer bacillus is often highly virulent to rabbits, guinea-pigs, and mice. Subcutaneous inoculation kills mice in thirty-six to forty-eight hours from septicemia. At autopsy there is a whitish exudate at the point of inoculation, the spleen is swollen and engorged with blood, kidney and liver show cloudy swelling and areas of necrosis. The bacilli may be cultivated from the heart's blood and all the organs. In rabbits and guinea-pigs subcutaneous injection requires large doses to kill, the animals dying of septicemia. With intrapleural inoculation death takes place from small doses, at autopsy the pleural cavity being filled with exudate containing the organisms which may also be obtained from the heart's blood. Peritonitis with glairy viscid effusion follows intraperitoneal inoculation. Rabbits are also sensitive to intravenous inoculations, which produce a septicemia with the usual changes in the internal organs.

Buchner[14] regards the Friedländer bacillus as antagonistic to *Bacillus anthracis*. Rabbits injected with anthrax on one side and with killed cultures of Friedländer on the other live longer than controls. V. Dungern[15] has shown that this occurs also with avirulent cultures and is due to the powerful tissue reaction with the active collecting of leukocytes.

**Distribution.**—The Friedländer bacillus is widely distributed in man under both normal and pathological conditions. It is present in a small proportion of cases of croupous pneumonia, 6 out of 83 (Weichselbaum), 8 to 10 per cent. (Honi). It rarely produces typical croupous pneumonia with lobar and lobular foci. The exudate is sticky and viscid. Central areas of softening and lung sequestra tend to form. The organisms are found in the exudates and in the blood-stream. Occasionally Friedländer bacilli are present in the blood during life (Pässler, Philipi, Howard).

In bronchopneumonia the Friedländer bacillus is frequently present. It was found by Netter in 23.08 per cent. in pure culture and in 22.64 per cent. of mixed infection, and by Eyre in six times in 62 cases in pure culture, with about the same incidence in mixed infections. It may be found in bronchopneumonia after diphtheria (Strelitz), after measles (Mooney and Honi). It may be present in the sputum in influenza, in phthisis, and in lung abscesses. It is found in ulcerative stomatitis (Bernabei), angina (Stooss), in a special form of stomatitis with large yellow or white patches (Nicolle and Hébert), in parotitis (Girode). It is found in many inflammations of the nose and nasal cavities (Howard), in otitis media (Zaufol), in otitis in nurslings (Kossel). It is almost constantly present in simple ozena or atrophic rhinitis and in many cases of hypertrophic rhinitis along with the rhinoscleroma bacillus. Found in the blood in general hemorrhagic septicemia by Howard and by v. Dungern and in puerperal infection by Howard. Present frequently at autopsy on individuals dead of various causes. Found in pleurisy, empyema, pericarditis, meningitis, brain abscesses, abscesses of kidney, cystitis, epididymitis, cholecystitis, rarely in appendicitis (Schermann). In many of these cases it may be a secondary invader from nose and throat. According to Netter the Friedländer bacillus is present normally in the buccal cavity in 4.5 per cent. Hasslauer found it thirteen times in 111 normal nasal cavities. Found in the pharynx in 10 to 20 per cent. of the cases examined by v. Lingelsheim and by Fürst. Outside the body organisms identified as the Friedländer bacillus have been found in the air by Pawlowsky, Uffelmann, and Welde, in the soil by Obrostowski and Jakowski, on floors by Emmerich, in dust by Solowjein and Zieleniew, in canal water by Mori, in mud by Nicolle and Hébert.

**Varieties.**—While many of the encapsulated bacilli derived from the nasal and pharyngeal

mucosa and from respiratory infections correspond culturally to the Friedländer bacillus as described above, many strains from these areas acidify and coagulate milk and ferment lactose, and thus would be regarded culturally as *Bacillus lactis aërogenes*. They have the same action upon animals as the typical *Bacterium pneumoniæ* of Friedländer. If we limit the identification of *Bacillus lactis aërogenes* to the encapsulated organisms in the intestines these strains from the respiratory mucosa would be called varieties of Friedländer. On the other hand, many of the encapsulated bacteria of the intestines may be derived from the nasal and pharyngeal surfaces, being swallowed with food and drink. The term *Bacillus lactis aërogenes* could, therefore, be applied both to the encapsulated organisms in the intestine and to those on the nasal and pharyngeal mucosa. It is probably better to regard all the encapsulated organisms from the nasal and pharyngeal mucosa and from respiratory infection as varieties of the Friedländer bacillus, reserving the name *Bacillus lactis aërogenes* for similar encapsulated organisms in the intestine.

### BACTERIUM RHINOSCLEROMATIS v. Frisch

Isolated from a peculiar hypertrophic rhinitis prevailing in Central Europe by v. Frisch[16] as early as 1882. The organism was first carefully studied by Paltauf and v. Eiselsberg.[17] It occurs in almost pure culture in the nasal mucosa of rhinoscleroma cases and also in the depths of the tissue. It is frequently associated with the Friedländer bacillus in rhinoscleroma. Paltauf considers it is the same as the Friedländer bacillus. Babes, however, describes a strain received from Kral's collection which has somewhat different reactions. As compared with other encapsulated bacilli this strain is quite rare. The following description is from a culture isolated from a case of the disease by Dr. Jobling in New York, which differs from the Friedländer bacillus and corresponds to Babes' description. There is no satisfactory evidence that the rhinoscleroma bacillus causes rhinoscleroma (Babes).

**Morphology.**—Short rods about 0.6 to 0.7 $\mu$ in breadth and 1 to 1.5 $\mu$ in length on plain agar twenty-four hours old. On dextrose agar the organisms are somewhat thicker and longer. Capsular substance abundant, often enclosing several individuals.

Non-motile. No spores.

**Stained** best after washing the films in running water, with gentian-violet, methylene-blue, or dilute carbolfuchsin. Gram-negative. Capsules stain easily, often by gentian-violet or safranin. Best demonstrated by the capsule stains of Welch, Hiss, or Huntoon. Metachromatic granules often present, demonstrated by Gram's stain followed by anilin safranin (Babes). The granules stain blue, the bacillary bodies red. In the tissues the bacteria are stained easily by fixation in osmic acid and staining with methyl violet, which bring out large, sharply outlined, delicately stained capsules.

**Cultural Characters.**—*Plain Agar Plates.*—Surface colonies in twenty-four hours round, regular, glairy, viscid, looking like large drops of dew, measuring 2 to 2½ mm. in diameter. Under low power dark, opaque, granular, nucleated, with entire edges. Deep colonies well developed, under low power usually oval or disk shaped. The surface colonies increase rapidly in size, when separated having a diameter of 7 to 8 mm. in six to eight days. The deep colonies in old cultures are distinctly lanceolate.

*Plain Agar Slant.*—Abundant, glairy, spreading growth in twenty-four hours, very viscid and sticky. Long threads of viscid material are brought away when the growth is touched with a platinum needle. In older cultures the growth spreads widely over the agar, especially in the lower portions, and the water of condensation is turbid and viscid.

*Plain Agar Stab.*—Abundant growth along line of inoculation in twenty-four hours, spreading moist and elevated on the surface about the line of puncture. In older cultures the line growth becomes thicker and more abundant, while the surface growth may spread to the wall of the tube.

*Dextrose Agar Plates.*—Large glairy, viscid, dew-drop colonies in twenty-four hours, measuring frequently 3 to 4 mm. Under low power opaque, granular, nucleated, with entire edges. Deep colonies lanceolate, ¼ to ½ mm. in diameter, under low power elliptical or disk-shaped. The surface colonies spread widely and after seven to eight days have heaped-up centers and thin peripheries. Deep colonies also increase in size. Agar highly acid in the first two to three days, the acidity gradually giving way to an alkaline reaction. In old plates the agar is deep blue in color. Occasionally the acidity remains permanently.

*Dextrose Agar Slant.*—Very abundant spreading growth in twenty-four hours, glairy, viscid, and stringy. Acidity high. No gas bubbles. In two to three days the surface of the

medium is covered by a glairy, viscid growth. The acidity may give way to an alkaline reaction or may remain permanent.

*Gelatin Plates.*—Colonies appear in two to three days, round, regular, and uniform, about ½ mm. in diameter. Under low power thicker in the center, thinner at the periphery, with

Fig. 104.—*Bacterium rhinoscleromatis* from plain agar twenty-four hours old, stained with gentian-violet.

Fig. 105.—*Bacterium rhinoscleromatis*, from dextrose agar twenty-four hours old, stained with gentian-violet.

entire edges. The colonies increase somewhat in size and tend to become glairy and viscid. Never as large as agar colonies. No liquefaction.

*Gelatin Stab.*—Delicate line and surface growth in two to three days, becoming more abundant in four to five days. In old cultures the line growth may be quite heavy with a spreading surface growth. No liquefaction.

*Blood-serum.*—Abundant, glistening, glairy, viscid growth in twenty-four hours. After two to three days this dries down somewhat and in old cultures forms a fairly abundant glistening expansion.

*Potato.*—Fairly abundant, moist, yellow growth in twenty-four hours. The growth increases markedly in three to four days and is moist, elevated, and cream-colored. In old cultures it dries down somewhat.

*Milk.*—Faint acid reaction in twenty-four hours, slightly increased in forty-eight hours. The acidity never becomes marked. No coagulation. In old cultures the acid may give way to an alkaline reaction, but does not do so regularly.

*Broth.*—Turbidity and viscid sediment in twenty-four hours with a slight ring growth. Turbidity and sediment increase in two to three days and are marked in old cultures. No scum formation.

*Peptone.*—Turbidity and viscid sediment in two to three days, increasing in old cultures. No scum.

*Dextrose Fermentation Tubes.*—Turbidity in bowl and arm in twenty-four hours, with a sediment in forty-eight hours. Reaction acid. No gas. Acid, no gas from saccharose. In lactose the growth is limited to the bulb, the arm remaining clear. No acid. Reaction alkaline.

**Pathogenic Action.**—But few observations have been made upon animals with this strain of encapsulated bacillus. Kraus[18] reports that cutaneous injection of white mice with scleroma bacilli produces cell changes like those seen in rhinoscleroma, the same reaction being produced with the Friedländer bacillus. In cats, usually immune to inoculation by members of the group, Streit[19] produced a pneumonia with scleroma bacilli.


1. Escherich: Die Darmbakterien des Säuglings. Stuttgart, 1886.
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3. Fasching: Sitzungsb. d. K. Akad. d. Wissensch. Math.-naturw. cl., c, Abt. 3.
4. Mori: Ztschr. f. Hyg., 1888, iv, 47.
5. Pfeiffer: Ztschr. f. Hyg., 1889, vi, 145.
6. Abel: Ztschr. f. Hyg., 1896, xxi, 89.
7. Howard: J. Exper. M., 1899, iv, 149. *Ibid.*, 1900–01, v, 139.
8. Perkins: J. Infect. Dis., 1904, i, 241. *Ibid.*, 1907, iv, 51.
9. Wilde: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1896, xx, 681.
10. Strong: Centralbl. f. Bakteriol. [etc.], 1899, xxv, 49.
11. Fürst: Centralbl. f. Bakteriol. [etc.], 1910, lvi, 97.
12. Hueppe: Mitt. a. d. K. Gsndhtsamte, 1884, ii, 1837.
13. Ford: Stud. Roy. Victoria Hosp., Montreal, 1901–03, i, No. 5, March.
14. Buchner: Berl. klin. Wchnschr., 1890, xxvii, 216.
15. v. Dungern: Ztschr. f. Hyg., 1894, xviii, 177.
16. v. Frisch: Wien. med. Wchnschr., 1882, xxxii, 970.
17. Paltauf and v. Eiselsberg: Fortschr. d. Med., 1886, iv, 617; 649.
18. Kraus: Wien. klin. Wchnschr., 1907, xx, 1422.
19. Streit: Centralbl. f. Bakteriol. [etc.], 1906, xl, 709. *See also* Abel and Hallwachs: Die Kapselbacillen. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, vi, 515.


### Bacterium Group VI

Liquefying, not producing acid or gas from carbohydrates.

*Bacillus Bookeri* Sternberg

Liquefying, producing acid and gas from carbohydrates.

*Bacillus cloacæ* Jordan.

*Streptobacterium fœtidum* Jacqué and Masay.

### Bacillus Bookeri Sternberg

Described originally by Booker[1] as Bacillus A from the dejecta in cases of summer diarrhea of infants. Named by Sternberg.[2] Found by Ford[3] in the intestinal contents of adults, and by Tissier[4] in summer diarrhea of children.

Description from personal observations and from Tissier.

**Morphology.**—Small pleomorphic bacilli about the size of *Bacilli coli*, measuring 0.4 to 0.5 $\mu$ in breadth and 1.5 to 2 $\mu$ in length. Short coccobacillary forms not uncommon. No spores.

Usually actively motile. Tissier's strains non-motile.

**Stains** well with anilin dyes. Gram-negative. Flagella peritrichous according to Bergey.[5]

**Cultivated** easily on ordinary media as a facultative anaërobe. Grows at 22° C. and best at 37° C. Can be transferred from broth cultures a month old and gelatin cultures four months old.

**Cultural Characters.**—*Plain Agar Plates.*—Surface colonies in twenty-four hours round, regular, $\frac{1}{2}$ to 1 mm. in diameter, like those of *Bacillus coli*. Under low power they are round with dense nuclei and entire margins. Deep colonies punctiform, under low power usually biconvex disks. In three to four days the surface colonies are 3 to 5 mm. in size with dense centers and thin margins, while the deep colonies are distinctly oval to the unaided eye. Bottom colonies spread widely and may be up to 1 cm. in diameter. After eight to ten days the colonies are somewhat larger, slightly yellow, looking like "café au lait" according to Tissier.

*Plain Agar Slant.*—Thick, abundant, smooth, glistening growth in twenty-four hours, somewhat resembling *Bacillus coli*. In cultures three to four days old the growth is increased in thickness and breadth, and in cultures ten to twelve days old it tends to become cream-colored.

*Plain Agar Stab.*—Abundant line and surface expansion in twenty-four hours with a ring growth about the wall of the tube. In old cultures the surface growth covers the entire medium. Tissier reports an abundant gas production in deep agar stabs, the gas arising from a decomposition of the proteins.

*Dextrose Agar Plates.*—Colonies grow slowly, appearing in two to three days as round, regular, elevated surface expansions 3 to 4 mm. in size, slightly yellowish in color. Under low power they are regular, opaque, with dense centers, dark margins, and thin entire edges. Deep colonies punctiform, under low power oval and disk-shaped. Older colonies are somewhat larger, but never grow as rapidly as in media without sugar. Agar alkaline in reaction.

*Dextrose Agar Slant.*—Thick, moist, fairly abundant growth in two to three days, slightly yellowish in color. In older cultures the growth is somewhat larger, but does not spread like that on plain agar. Agar alkaline in reaction.

*Gelatin Plates.*—Colonies appear in two to three days, yellowish, irregular, with broken feathery edges. Under low power they show dense skein-like centers and irregular feathery edges. Deep colonies punctiform, under low power looking like densely wound balls of wool. The gelatin is slowly liquefied about the colonies which lie in zones of fluid gelatin nearly 1 cm. in diameter. In old plates the gelatin is completely liquefied.

*Gelatin Stab.*—Faint line growth appears in twenty-four hours with a slight surface cupping. In three to four days there is an abundant cup-shaped liquefaction. The gelatin is completely liquefied in ten to twelve days. It takes on a mahogany-brown color (Tissier).

*Blood-serum.*—Thick, moist, yellowish growth in twenty-four to forty-eight hours. After three to four days the growth is increased in extent and somewhat sunken. No definite liquefaction.

*Potato.*—Fairly abundant expansion in two to three days, not very thick, yellowish, or chestnut-colored. In older cultures the color is distinctly brownish, probably from the starch in the potato.

*Litmus Milk.*—Alkaline reaction in twenty-four hours with some clearing in the upper portion. In two to three days the litmus is reduced and the proteins partially digested with a clear zone above and a soft coagulum below. After ten to twelve days the soft coagulum may be entirely digested, the serum turning somewhat yellowish. The casein is often completely used up by the organisms.

*Broth.*—Abundant turbidity and sediment. No surface film, but a well-marked ring growth.

*Peptone.*—Abundant turbidity and sediment with a delicate fragile scum.

**Smith Fermentation Tubes.**—*Dextrose.*—Dense turbidity and sediment in bowl. Arm clear. Good line of demarcation. No action on the sugar. Reaction alkaline.

No fermentation of other carbohydrates, including levulose, galactose, saccharose, lactose, mannite, dulcite, and starch.

All media containing starch turn brown and media containing tyrosin take on a rose color.

Tissier has observed that *Bacillus Bookeri* attacks proteins and albuminoids. In mixtures of peptone and animal or vegetable albumin it uses up the peptone and amino-acids, but leaves the albumin. Its attack on albumoses, peptones, and amino-acids is not so intense as that of *Proteus vulgaris*. Thus the Biuret reaction does not disappear in peptone solutions. Indol and phenol are not produced. Tissier believes that the organism has a simple proteolytic ferment like that of other aërobes which dissociates the albuminoid molecule slowly to ammonia.

**Pathogenic Action.**—Tissier's cultures were virulent to mice, ½ c.c. of a broth culture given subcutaneously killing them in twelve hours. Intraperitoneal injection of 1 c.c. killed guinea-pigs in a few hours, but subcutaneous injection merely made them ill for a short period. Killed cultures and filtered cultures were fatal to guinea-pigs by intraperitoneal administration, but produced only a transient malaise, lasting three to four days, on subcutaneous introduction. The action is like that of *Proteus vulgaris*, but less intense.

Tissier believes that this species is responsible for digestive disturbances in children, especially in those cases without fever, with slight colic, depression, loss of weight, and diarrhea with eight to ten soft, viscid, glairy stools daily.

1. Booker: Tr. 9. Internat. Med. Cong., 1887, iii, 598.
2. Sternberg: Manual of Bacteriology. 1892, p. 392.
3. Ford: Studies from the Royal Victoria Hospital, 1901–03, i, No. 5.
4. Tissier: Ann. de l'Inst. Pasteur., 1920, xxxiv, 684.
5. Bergey: p. 236.

### BACILLUS CLOACÆ Jordan

Found originally by Jordan[1] in sewage. Present description based on Jordan's original statements, and on a number of personal isolations from water and sewage.

**Morphology.**—Short, rather thin bacilli measuring 0.4 to 0.5 $\mu$ in breadth and 1 to 2 $\mu$ in length. They are, in general, somewhat more delicate than *Bacillus coli*. Slightly enlarged on dextrose agar.

**Motility** very active in young and especially in freshly isolated cultures. Flagella peritrichous (Bergey). In old cultures and in many strains from water motility is difficult to demonstrate and may be lost.

**Stains** easily by the usual anilin dyes. Gram-negative.

**Cultivated** without difficulty on the ordinary media. Grows best at 37° C., but well at 22° C.

**Cultural Characters.**—*Plain Agar Plates.*—Surface colonies round, regular, and opaque in twenty-four hours, 2 to 3 mm. in diameter. Under low power they are dense and opaque with corrugated centers, thin margins, and entire edges. Deep colonies punctiform, under low power usually round or oval. The surface colonies increase fairly rapidly in size and in two to three days are ½ to 1 cm. in diameter, rather thick, often with dense centers and thin margins. The deep colonies when older are round or biconvex disks to the unaided eye. In ten to twelve days the surface colonies often show thick central masses and irregular margins with radiating prolongations. Occasionally irregular masses of daughter colonies appear about the original surface colonies.

*Plain Agar Slant.*—Thick, moist, regular, whitish, non-spreading growth in twenty-four hours, a little like that of *Bacillus coli*, but usually somewhat heavier.

The growth increases rapidly in breadth and thickness and frequently extends over the entire agar surface. It is nearly always thick and does not show the creeping edges of *Proteus vulgaris*, although the edges may be somewhat crenated. Old cultures often become slightly yellowish in color, especially in the lower portions.

*Plain Agar Stab.*—Abundant line growth and surface expansion in twenty-four hours. In two to three days the line growth is distinctly thicker and the surface expansion wider. In cultures ten to twelve days old the surface growth may be very heavy and wide, reaching the walls of the tube. It is often slightly cream-colored.

*Dextrose Agar Plates.*—Surface colonies grow rapidly, appearing in twenty-four hours as round, regular, elevated expansions often 2 to 3 mm. in size when separated. Under low power they show dark central heaped-up nuclei, rather thin margins, and entire edges. Deep colonies punctiform, under low power round, oval, or biconvex disks. The agar shows an acid reaction and bubbles of gas. When the plates are thickly seeded the acidity gives way to alkalinity very early and sometimes the agar is alkaline in twenty-four hours. In three to four days the surface colonies are larger, 4 to 5 mm. in diameter, round, and regular, tending to be elevated rather than flat like dextrose agar colonies of *Bacillus coli*. They usually show rather thicker central masses and thin margins. Deep colonies when older are considerably larger and somewhat irregular in shape, under low power appearing as broken, irregular disks, or moruloid masses. By the end of three to four days the acid reaction is entirely overcome by alkali production and the agar is alkaline and deep blue in color. Older surface colonies (ten to twelve days) may be 6 to 8 mm. in diameter.

*Dextrose Agar Slant.*—Thick, moist, whitish to cream-colored, non-spreading growth in twenty-four hours, a little like that of *Bacilli coli*, but heavier. The agar show an acid reaction with bubbles of gas in the water of condensation. Alkali production appears quickly and sometimes in twenty-four hours the acidity begins to lessen and the agar shows an alkaline reaction in certain areas. In two to three days the growth is very heavy, whitish to cream-colored. By this time the acidity is entirely overcome and the agar deep blue.

Fig. 106.—*Bacillus cloacæ* from plain agar twenty-four hours old, stained with carbolfuchsin.

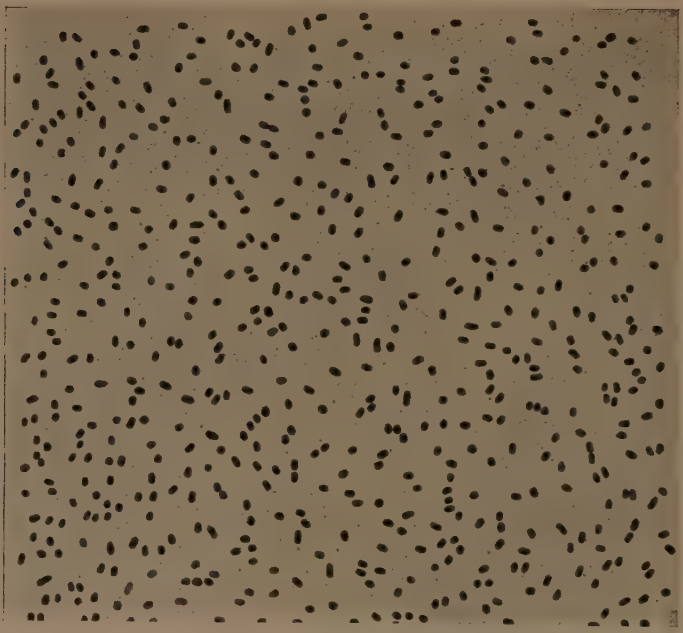

Fig. 107.—*Bacillus cloacæ* from dextrose agar, twenty-four hours old, stained with carbolfuchsin.

*Dextrose Agar Stab.*—Rapid growth in the depths and on the surface, the agar turning acid and being fragmented by gas bubbles. In two to three days the acidity is entirely overcome and the reaction alkaline.

*Gelatin Plates.*—Colonies appear in two to three days, ½ to 1 mm. in size. Under low power they are round, regular, opaque, with thin margins and entire edges. Older colonies are raised, elevated, circumscribed, 1 to 2 mm. in diameter. They are usually thicker and denser than gelatin colonies of *Bacillus coli*. In actively liquefying strains liquefaction may begin in two to three days and the colonies lie in small saucers of liquid gelatin. In eight to ten days the colonies often are surrounded by zones of liquid gelatin, ¾ to 1 cm. in diameter. In old plates the gelatin is entirely liquefied. Daughter colonies are not uncommon in gelatin plates about the original colonies.

*Gelatin Stab.*—Faint line and surface growth in twenty-four hours. In three to four days the line growth may be distinctly heavier and a slight surface cupping of the gelatin appear. The liquefaction often extends rapidly, usually along the line of inoculation from the surface downward. Occasionally a stratiform liquefaction appears. The gelatin may be completely liquefied in twelve to fifteen days.

*Blood-serum.*—Raised whitish or cream-colored growth in twenty-four to forty-eight hours. The growth increases in extent and may become quite heavy in old cultures, but does not tend to spread. No liquefaction in cultures observed by the present author.

*Potato.*—Abundant, moist, pale yellow growth in twenty-four hours. The growth may become much more abundant in old cultures and be reddish-brown in color. Occasionally gas bubbles appear in the fluid beneath the potato.

*Litmus Milk.*—Highly acid in twenty-four hours with a reduction of the litmus in the depths. Coagulation may occur spontaneously or only on heating. Milk proteins not digested.

*Broth.*—Turbidity and sediment. No scum.

*Peptone.*—Turbidity and sediment. No scum.

**Smith Fermentation Tubes.**—*Dextrose.*—Turbidity and sediment in the bowl in twenty-four hours, the turbidity extending up into the closed arm. Rapid fermentation of the dextrose, 1 to 1½ inches of gas collecting in twenty-four to forty-eight hours. The reaction of the broth is acid, but may give way to alkali in the bulb in two to three days. About three-quarters of the gas is absorbed by KOH, the balance explosive. Gas formula $\frac{H}{CO_2}=\frac{1}{2-3}$. Acid and gas production also with levulose, galactose, maltose, saccharose, lactose, arabinose, raffinose, mannite, sorbite, and dextrin.

Methyl red reaction negative.

Voges-Proskauer positive.

Nitrates reduced to nitrites.

Indol variable.

**Distribution.**—*Bacillus cloacæ* is widely distributed in nature. It occurs frequently in sewage, in water, in oysters, and in sewage-polluted soil. It is not definitely determined whether it is present in the intestinal tract of man, but it is present in the dejecta of animals. The cultures of organisms called "non-fecal coli" or high ratio organisms are in many instances non-liquefying *Bacillus cloacæ*.

Jordan: Rep. Mass. State Board of Health, Part XI, 1890, p. 836.

### Non-liquefying Bacillus cloacæ

In many instances strains of *Bacillus cloacæ* liquefy gelatin very slowly and occasionally strains occur which show no liquefaction of gelatin even after many days, observation. These have already been mentioned by Jordan. In the present author's study of this group two strains were encountered which failed to liquefy gelatin. One was from the intestinal contents of a horse and the other from spring water. They showed the typical morphology, motility, and cultural reactions with the exception of gelatin. They fermented dextrose, saccharose, and lactose with acid and gas, 2 to 3 parts of the gas being absorbed by KOH, the balance being explosive.

### Streptobacterium fœtidum Jacqué and Masay

Isolated by Jacqué and Masay[1] from sputum, meningeal pus, pleural fluid, abscesses, etc.

**Morphology.**—Short bacillus with rounded ends, really a cocco-bacillus. Often in very long chains in bouillon. It resembles the plague bacillus. Very motile. No spores.

Stains by usual anilin dyes. Gram-negative. Often shows polar staining.
**Culture.**—Grows at ordinary temperatures, and as low as 10° C.
Optimum temperature, 37° C.
Facultative anaërobe.
All cultures give off a very fetid odor.
*Broth* at 37° C. Turbidity in a few hours, considerable sediment.
*Glucose Broth.*—Abundant gas.
*Maltose Broth.*—Abundant gas.
*Serum.*—Rapid growth like that in broth.
*Agar Slant.*—Thick, abundant, rapidly spreading growth over entire surface.
*Maltose Agar.*—Rapid growth slower than ordinary agar.
*Blood Agar.*—Rapid growth with hemolysis.
*Gelatin.*—Abundant growth along line of inoculation with liquefaction in form of glove finger. Eventually gelatin completely liquefied.
*Potato.*—Abundant surface growth.
*Coagulated Serum.*—Cream-colored layer with rapid liquefaction.
Destroyed at 58° C. in half an hour.
**Virulent** to white rats, subcutaneous inoculation producing a local abscess or fatal septicemia. Intraperitoneal inoculation produces localized peritonitis or septicemia. Equally virulent to rabbits, guinea-pigs, and white mice.
Virulence lost in old cultures.
Filtered broth cultures toxic in large doses (10 c.c.) to rabbits, guinea-pigs, rats, and mice. Animals (white rats) vaccinated with killed cultures develop agglutinins and the Bordet-Gengou reaction.

Jacqué and Masay: Centralbl. f. Bakteriol. [etc.], 1. Abt., lxii, 1912, 180.

## Tribe 5. Lactobacilleæ Com.

Rods, often long and slender, Gram-positive, non-motile, without endospores. Usually produce acid from carbohydrates, as a rule lactic. When gas is formed it is $CO_2$ without $H_2$. The organisms are usually somewhat thermophilic. As a rule micro-aërophilic; surface growth on media poor.

### GENUS 7. LACTOBACILLUS Beijerinck

**Generic Characters Those of the Tribe.**—The genus Lactobacillus includes a large number of micro-organisms isolated from a variety of sources which have certain morphological, tinctorial, and cultural features in common. They are usually long and slender Gram-positive rods which tend to grow out into chains and long threads. Certain of them show remarkable variations in form and size, and one species at least if not more exhibits the phenomenon of true branching. The majority grow better anaërobically than aërobically and some of them are obligate anaërobes. They are usually cultivated with difficulty on ordinary media, growing only when acid, sugar, whey, or beerwort are added. It is frequently necessary to use acid media in their original isolation. They usually produce large quantities of acid from carbohydrates, much more than the ordinary lactic acid streptococci and micrococci from milk or the more common intestinal bacteria. Some forms produce gas as well as acid, this gas being carbon dioxid. The majority of the true lactic acid bacteria grow at high temperatures, 45° to 50° C., and some of them have been described originally as thermophils because of this character. They do not produce spores nor are they endowed with motility. Occasionally spore-bearing or motile forms have been described in this group, but such organisms should properly be classed with the bacteria or the bacilli. For convenience of description and identification the genus may be divided into environmental and parasitic lactobacilli.

**Environmental Lactobacilli.**—This group includes the true lactic acid organisms found in the various types of buttermilk used so largely in the Orient and those found in the different types of cheese. The first organism of this type to be described is probably *Bacillus caucasicus* of Freudenreich,[1]

observed originally by Kern[2] in the milk product known in Russia as kefyr. This is evidently the same species as *Bacterium caucasicum* or *Lactobacillus caucasicus* isolated by Beijerinck,[3] also from kefyr. With this organism, a yeast known as *Saccharomyces keferi*, and two varieties of streptococci Freudenreich produced in sterile milk a fermentation like that caused by kefyr grains in milk.

Somewhat later Rist and Khoury[4] made a thorough investigation of the Egyptian milk product known as leben rafb or leben. This is known in Arabia as leben and in Greece and Turkey as yaourte. Leben is a clotted milk of about the consistency of cottage cheese. The curd is white and flocculent and expresses only a small quantity of serum or whey. It is eaten only when reasonably fresh, since in two to three days the acidity increases so that it cannot be used as food. Rist and Khoury[5] have identified different species which take part in the fermentation of milk which results in leben, *Streptobacillus lebensis*, *Bacillus lebensis*, *Diplococcus lebensis*, *Saccharomyces lebensis*, and *Mycoderma lebensis*. Some of these organisms are closely similar to the species found in kefyr. Some three years after the work of Rist and Khoury Grigoroff[5] in Massol's laboratory studied the Bulgarian milk product known as yoghurt. This is more fluid in consistency than leben and is usually known as Bulgarian buttermilk. In many instances it is sufficiently fluid to be employed as a drink. Grigoroff found three species in this product, a *Bacillus* A, a *Micrococcus* B, and a *Streptococcus* C. With the combination of these three organisms he produced a buttermilk like the Bulgarian variety. *Bacillus* A is usually regarded as the true type of *Bacillus Bulgaricus*, subsequently investigated and apparently named by Cohendy.[6] Mazé,[7] however, in an investigation of yoghurt, found two types of bacteria which he regarded as identical with two of Rist and Khoury's organisms, *Streptobacillus lebensis* and *Streptococcus lebensis*. Finally Düggeli[8] found in the Armenian milk product known as mazun a bacillus not unlike *Bacillus Bulgaricus*, its protoplasm, however, showing granules intensely staining with methylene-blue. This was later isolated and described at length as *Bacterium mazun* by Weigmann, Gruber, and Huss,[9] who also obtained constantly a spore-bearing peptonizing bacillus, *Bacillus mazun*, which closely resembles *Bacillus albolactis*. Similar granular bacilli were observed by Grixoni[10] in the Sardinian milk product known as gioddu, found only in association with Gioddu yeasts, and by Luerssen and Kuhn[11] in commercial buttermilk along with the true *Bacillus bulgaricus*. This latter organism is known usually as the "Körnchenbacillus." In addition to these true lactic acid organisms obtained from buttermilk and clotted milk closely related bacteria are active in the ripening of certain varieties of cheese, particularly that type known as Emmentaler cheese. The species isolated exhibit great diversity in their morphology and cultural reaction, and are associated with many other bacteria, micrococci, and streptococci. Thus several species of bacteria belonging to the lactic acid group have been isolated from cheese by Leichmann and Bazerewski,[12] while Freudenreich and Thöni[13] have found a variety of micrococci and bacilli. Other types of bacteria found in cheese with the lactic acid organisms are those which produce propionic acid described by Freudenreich and Jensen.[14] This interesting field of research belongs properly to industrial or milk bacteriology and need not be considered in a book on medical or public health bacteriology.

**Parasitic Lactobacilli.**—The first organisms of this group to be observed is the species known usually as the Oppler-Boas bacillus or the Boas-Oppler bacillus and described by Oppler[15] in 1895. There has been much discussion

in regard to this organism, caused chiefly by the difficulty of satisfactory isolation. Subsequently a species known as *Bacillus acidophilus* was found and reported in the same year by Moro[16] and by Finkelstein.[17] This species was investigated thoroughly by Tissier,[18] who described a closely related form, *Bacillus exilis*. At the same time he reported at length upon possibly the most important member of the group, *Bacillus bifidus communis*, or *Bacillus bifidus*, as it is commonly known. Finally an interesting gas-producing lactic acid bacillus has recently been isolated by Torrey and Rahe.[19] The parasitic lactic acid bacteria are often called "acidophilic" because of their ability to grow in acid media. As pointed out by a number of authors, especially by Kendall,[20] these organisms are not really especially attracted by acid, but are acid-resistant, in consequence of which they can be isolated from mixtures by the use of acid media in which ordinary bacteria do not grow. For this reason the term "aciduric" introduced by Kendall is more strictly applicable. From time to time efforts have been made to identify the lactic acid organisms in the intestine with organisms in the environment. Thus far none of these identifications can be regarded as satisfactory, although their early appearance in the stools suggests an environmental origin.

At the present time we have no satisfactory classification of the lactic acid bacteria. The various species may, therefore, be described independently.

1. Freudenreich: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1897, iii, 135.
2. Kern: Bull. Soc. Imp. des naturalistes de Moskow, 1881, No. 3.
3. Beijerinck: Arch. neérl. d. sc. exactes [etc.], 1889, xxiii, 428.
4. Rist and Khoury: Ann. de l'Inst. Pasteur., 1902, xvi, 65.
5. Grigoroff: Rev. méd. de la Suisse Rom., 1905, xxv, 714.
6. Cohendy: Compt. rend. Soc. de biol., 1906, lx, 558.
7. Mazé: Ann. de l'Inst. Pasteur., 1905, xix, 378.
8. Düggeli: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xv, 577.
9. Weigmann, Gruber, and Huss: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1907, xix, 70.
10. Grixoni: Cited by Bertarelli, Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xv, 750.
11. Luerssen and Kuhn: Centralbl. f. Bakteriol. [etc.], 2 Abt., 1908, xx, 234.
12. Leichmann and Bazerewski: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1900, vi, 245.
13. Freudenreich and Thöni: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1903, x, 305.
14. Freudenreich and Jensen: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xvii, 529.
15. Oppler: Deutsche med. Wchnschr., 1895, xxi, 73.
16. Moro: Wien. klin. Wchnschr., 1900, xiii, 114.
17. Finkelstein: Deutsche med. Wchnschr., 1900, xxii, 263.
18. Tissier: Recherches sur la flore intestinale des nourrissons. Paris, 1900.
19. Torrey and Rahe: J. Infect. Dis., 1915, xvii, 437.
20. Kendall: J. Med. Research, 1910, xxii (n. s. xvii), 153.

*Environmental*

Type species *Lactobacillus caucasicus* (Kern) Beijerinck.
*Kurmys bacillus.*
*Streptobacillus lebensis* Rist and Khoury.
*Bacillus lebensis* Rist and Khoury.
*Bacillus bulgaricus* Grigoroff.
*Streptobacillus* C Grigoroff.
*Bacillus sardous* Grixoni.
*Bacterium mazun* Weigmann, Gruber, and Huss.
The Körnchenbacillus of Luerssen and Kuhn.
*Diplococcus lebensis* Rist and Khoury.
*Micrococcus* B of Grigoroff.
*Streptothrix* from Dadhi, Chatterjee.

*Parasitic*

Type species *Lactobacillus Boas-Oppleri* (Boas and Oppler).
*Bacillus acidophilus* Moro.
*Bacillus exilis* Tissier.
*Bacillus acidophil-aërogenes* Torrey.

*Anaërobic*

*Bacillus bifidus communis* Tissier.

### Lactobacillus Caucasicus (Kern) Beijerinck

First isolated from milk in the Caucasus and described by Kern[1] as *Dispora Kaukasica*. Named *Bacillus Kaukasicus* by Kruse.[2] Opinions differ as to whether this is a definite species, since kefyr contains several kinds of molds, sarcinas, and a number of sporulating bacteria. According to Kruse, Kern's organism was a slowly motile bacillus measuring 0.8 by 3.2 to 8 $\mu$, with round spores 1 $\mu$ in diameter at both ends. Cultivated only in fluid media.

In the opinion of von Freudenreich[3] kefyr grains contain 2 types of streptococci, yeasts, and a bacillus which was probably the one seen by Kern. This is an organism with bipolar staining (not spores) which appears in lactose broth as a straight bacillus with round ends and refractile granules at either end. It forms minute colonies on the surface of whey agar and grows well in the depth of agar stabs. No growth on gelatin plates or lactose gelatin plates. After long laboratory cultivation growth appears in a gelatin stab. Colonies on whey agar small, flat, granular, round to the naked eye. Under low power round or irregular, whitish, and granular. No growth at 35° C. in broth. Lactose broth shows a little growth at 22° C. At 37° C. development better. Reaction strongly acid. Acid reaction in milk. No clot. Taste sour and astringent. No growth on potato.

Beijerinck[4] states that the bacilli found in kefyr are very difficult to cultivate, growing only after two to three weeks on whey gelatin. They grow best at high temperatures, 40° to 50° C. and form lactic acid from glucose, lactose, saccharose, and maltose.

1. Kern: Bull. Soc. Imp. des naturalistes de Moskow. 1881, No. 3.
2. Kruse: Die Mikroorganismen (Flügge), 1896, ii, 270.
3. von Freudenreich: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1897, iii, 135.
4. Beijerinck: Arch. néerl. d. sc. exactes [etc.], 1889, xxiii, 428.

### The Kumysbacillus

Found in kumyss in association with yeasts and *Bacillus acidi lactici*. Cultivated by the Liborius method in deep solid media by Schipin.

Growth only in the deeper portions of stab cultures. Obligate anaërobe. Gram-positive bacillus. No spores. Cultivated in an atmosphere of hydrogen. Grows best on acid and on sugar gelatin, fairly well on ordinary and on alkaline gelatin. No liquefaction. Slight development on plain agar, sugar agar, and glycerin agar. Moderate growth in blood-serum. No growth in broth. No apparent growth in milk at room temperature under aërobic conditions. At 37° C. it clots milk in the form of a thick mass without expression of whey, also when exposed to the atmosphere. Optimum temperature 20° to 30° C. Killed at 57° C. in one-half hour, at 60° C. in ten minutes. Gas production in gelatin and blood-serum. In mares' milk produces $CO_2$ but $H_2S$ is not found. According to Schipin this bacillus breaks up lactose with the production of lactic acid and alcohol and also has the ability to peptonize casein. It plays the principal rôle in the fermentation of milk which results in kumyss, the favorable conditions for its multiplication being furnished in the yeasts and by *Bacillus acidi lactici*.

Schipin: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1900, vi, 775.

### Streptobacillus lebensis Rist and Khoury

Described originally by Rist and Khoury from the fermented milk known in Arabia as leben and in Greece and Turkey as yaourte.

**Morphology.**—Straight rods with square ends, non-motile. No flagella, no capsules, ½ by 6 to 8 mm. in size. In liquid media long chains appear, showing clear space between the elements when stained with carbolfuchsin. Often in parallel bundles. In chains often long rods, 18 μ long. Old cultures show long threads, thick and embossed. Protoplasm becomes granular and stains irregularly. Often twisted masses of curved rods appear. Filamentous forms occur in milk.

**Stains** by usual dyes. Living forms Gram-positive, dead forms Gram-negative.

Grows both aërobically and anaërobically.

Best isolated by cultures in milk, which is the most favorable medium, and then transferring to sugar agar or sugar broth. Somewhat difficult to accustom to laboratory cultivation. Lives eight days or more in glucose agar or glucose broth at ordinary temperature. At 37° C. grows more rapidly, but dies out sooner. Best kept alive in milk, transfers every month necessary. Lives only few days in deep sugar gelatin.

**Cultural Characters.**—*Glucose or Lactose Agar.*—Surface colonies in forty-eight hours at 37° C. appear as small flat areas with irregular contours and irregular surfaces, almost invisible by reflected light, pale gray or bluish by transmitted light. They are translucent and give an appearance as if their gray surfaces were traversed by bright striations. The general disposition of the colonies resembles that of melting snowflakes. Under low magnification (Zeiss Obj. m/m, Aperture 0.30, Ocular No. 2) the colonies are brownish in the center and seem to be made up of irregular curved hairy convolutions. They grow slowly, are usually 1.5 to 2 mm. in diameter, rarely 4 mm.

*Deep Glucose Agar* (Liborius Tubes).—Colonies extremely delicate, looking as if they were dissolving in the medium. They are transparent with ill-defined contours, here the same color as the agar with a "snowy" aspect. No growth on plain agar.

No growth in *plain gelatin.* Feeble growth in *lactose gelatin.* Along line of puncture isolated pin-point colonies appear, dense, white, opaque. No liquefaction.

No growth on *potato.*

No growth in *plain broth.* In *glucose* or *lactose broth* a turbidity, with later a coarse flocculent sediment.

Little or no growth in *filtered whey.* No gas production.

*Milk* coagulated in six hours at 37° C. High acidity from action on lactose. In a series of experiments to determine the amount of lactic acid which will coagulate milk at 37° C. it was determined that coagulation begins at about 0.558 per cent. acidity expressed in terms of lactic acid, and is complete at 0.720 per cent. *Streptobacillus lebensis* coagulates milk with an acidity of 0.26 per cent., while an acidity of 0.342 alone does not coagulate. The lactic acid clot is made up of flocculent masses separated by serum. The streptobacillus clot appears as a single block of material. In milk containing sodium carbonate to neutralize the acidity this organism clots the milk in less than twenty-four hours at 37° C. It thus produces a true rennin ferment. No casease. No digestion of the clot in the milk.

Rist and Khoury: Ann. de l'Inst. Pasteur., 1902, xvi, 65.

### BACILLUS LEBENSIS Rist and Khoury

Described by Rist and Khoury from leben.

**Morphology.**—Fine bacillus, 2 to 6 μ long, often slightly curved, Non-motile. No cilia. No capsules.

Appears usually as single elements, arranged in masses when taken from solid media. No chains, but in old cultures a few forms are end to end. No changes in morphology on different media. No swollen forms. In whey appears granular like the tubercle bacillus, which it resembles in size and form. No spores.

It is more variable than the *Streptobacillus lebensis* and lives longer on artificial media.

**Staining Reactions.**—Retains Gram's stain when young. Old dead organisms decolorize. In relatively young cultures, forty-eight to seventy-two hours, one can find both Gram-negative and Gram-positive forms.

*Grows* aërobically and anaërobically, but develops only on sugar media.

**Cultural Characters.**—*Glucose or Lactose Agar.*—Surface colonies in twenty-four hours at 37° C. small, almost colorless, transparent points like dew-drops. In forty-eight hours they are characteristic convex areas almost transparent, slightly yellowish-white, with round edges and humid reflecting surfaces. Viewed by transmitted light they are porcelain-like, bluish, iridescent, and often highly refractile. They attain a diameter of 2 to 3 mm., but do not grow beyond this size. Under low power young colonies resemble drops of dew made up of small colorless circular masses. Older colonies become yellow, particularly in the centers. About these yellow centers lie thin, colorless borders with granular edges. The entire surface of the colonies is finely granular. Occasionally the colonies seem to be composed of concentric striations like onions or grains of starch.

*Deep Glucose Agar.*—Opaque yellowish spherical or disk-shaped colonies with smooth edges.

*Lactose Gelatin.*—Along line of inoculation a continuous series of white, round, very regular colonies as if fastened to a stem. No liquefaction.

*Lactose or Glucose Broth.*—Abundant turbidity in twenty-four hours, producing an aspect "chaloyant" on shaking. Lactic acid produced.

*Whey.*—Excellent growth with a turbidity and an abundant sediment in forty-eight hours. High acidity, about .216 per cent. in terms of lactic acid in twenty-four hours.

*Milk.*—Excellent growth. No coagulation.

*Potato.*—No growth.

Rist and Khoury: Ann. de l'Inst. Pasteur., 1902, xvi, 65.

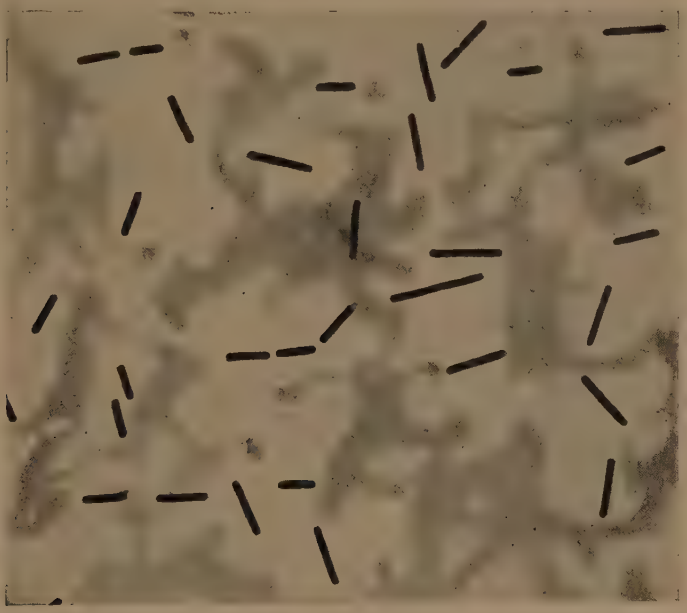

Fig. 108.—*Bacillus bulgaricus* from two weeks' culture in milk, stained with gentian-violet.

### Bacillus Bulgaricus Grigoroff

Obtained from Bulgarian buttermilk, known as yoghurt by Grigoroff, and described as *Bacillus* A. Isolated by transferring some of the fermented milk to lactose agar from which deep glucose agar tubes were made according to the method of Liborius as modified by Veillon.

**Morphology.**—Long rods in single elements or in thread-like chains. Non-motile.

**Staining Reactions.**—Stains by the usual dyes. Gram-positive.

**Cultural Reactions.**—Aërobe and facultative anaërobe. Grows poorly on ordinary media. Grows well on media containing sugars.

*Glucose Broth.*—Turbidity in twenty-four hours, with a white deposit at the bottom of the tube. At the end of three to four days the broth clears and has an acid reaction.

Lactose fermented just as glucose.

*Lactose Gelatin.*—No growth.

*Potato.*—No growth.

*Milk.*—Acidification and coagulation in ten to fourteen hours at 37° C. This is due to lactic acid. In milk kept neutral or alkaline by sodium carbonate coagulation does not occur. No digestion of the coagulum.

No indol. Produces true denitrification. In a medium containing 1 gram peptone, 1 gram potassium nitrate in 100 c.c. water fermentation occurs with a liberation of nitrogen.

In sterilized milk this organism produces a buttermilk like the true Bulgarian product, but a trifle more acid. It resists heat, but does not produce spores.

Optimum temperature 45° C. At this temperature milk is coagulated in five hours. At 37° C. coagulation occurs in ten to twelve hours. It grows at 50 °C. and coagulates in ten to twelve hours. Destroyed by 60° C. in an hour.

Ferments also maltose, levulose, mannite, and saccharose. Saccharose is first inverted, reducing Fehling's solution.

Does not attack rhamnose, dulcite, or sorbite. Produces inactive lactic acid and a trace of alcohol as shown by the iodoform test.

With Micrococcus B and Streptococcus C produces true Bulgarian buttermilk.

Grigoroff: Rev. méd. de la Suisse Rom., 1905, xxv, 714.

**Varieties of Bulgarian Bacilli.**—Following the work of Grigoroff a number of organisms have been isolated and described as Bulgarian bacilli which have in common the essential features mentioned by Grigoroff, but which differ in minor particulars. There is, therefore, quite a group of these organisms. Thus Cohendy[1] worked with a lactic ferment to which he gave the name "bacille bulgare." This organism was a large non-motile Gram-positive bacillus measuring 2 by 20 $\mu$ and growing out into sinuous chains containing two to ten elements. It could be cultivated aërobically, but grew better as an anaërobe. In deep agar it produced small thick lenticular umbilicated colonies 1 mm. in diameter in the anaërobic zone. It coagulated milk in eighteen hours at 37° C., did not attack the casein, but saponified the fats in the milk, producing a disagreeable taste like candle grease. This latter action was due to a lipase. In carbohydrates Cohendy's bacillus formed four to five times as much acid as ordinary lactic acid bacteria. Thus the acidity produced in ten hours at 36° C. was equal to 18.5 gm. in terms of sulphuric acid and to 32.5 gm. in terms of lactic acid for each liter of milk. Other organisms produced no more than 10 grams of acid in terms of lactic acid under the same conditions. This organism fermented glucose, levulose, saccharose, lactose, and maltose, and was closely similar to Grigoroff's *Bacillus* A.

Subsequently Bertrand and Weissweiller,[2] working with Cohendy's organism, concluded that butterfat and casein were decomposed to some extent by the growth in milk and that the acids evolved were a mixture of lactic and volatile fatty acids. The lactic acid itself was a mixture of dextro- and levo-rotatory, the dextro-rotatory predominating. Still later Bertrand and Duchacek[3] stated that Cohendy's bulgarian bacillus fermented dextrose, galactose, levulose, lactose, and mannite, but did not attack saccharose, maltose, arabinose, xylose, and sorbite. The fermentation of maltose originally noted by Cohendy was not observed by Bertrand and Duchacek.

In 1909 Heineman and Hefferan[4] investigated a number of different strains of *Bacillus bulgaricus* and closely related lactic acid organisms. They isolated bacilli of this type by the inoculation of material into milk which was incubated at high temperature. Plates were then made on a medium containing 0.5 per cent. glacial acetic acid and 2 per cent. glucose. They found organisms which they identified as *Bacillus bulgaricus* in a variety of locations, in sour and aromatic foods, in foods used for cattle, in market milk, in various fermented milks, in manured and non-manured soils, in normal saliva, in normal gastric juice, in gastric juice when hydrochloric acid was absent, in human, bovine, and horse feces. The growth of these organisms was best in milk or in media containing whey, peptone, or glucose. Optimum temperature 42° to 44° C. No growth at room temperature. The acidity in milk was more than 3 per cent., the acid consisting of 6 per cent. volatile acid and 94 per cent. optically inactve lactic acid. Both casein and fat were partially decomposed. As a result of their investigations Heineman and Hefferan concluded that the *Boas-Oppler bacillus*, *Bacillus panis fermentati*, *Streptobacillus lebensis*, and *Leptothrix buccalis* were all identical with *Bacillus bulgaricus* and that *Bacillus acidophilus* and *Bacillus bifidus* were possibly the same. They further suggested that *Bacillus bulgaricus* in the saliva might produce excessive acid and cause dental caries and that its presence in the intestine was not definite evidence of its transplantation on the mucosa in feeding experiments. Valuable as is this work of Heineman and Hefferan, it should be noted that these investigators did not employ a sufficiently large number of cultural criteria to enable them to differentiate between the various members of the group of true lactic acid organisms. This is made evident by the investigations of White and Avery[5] who studied a large number of lactic acid organisms from Bulgarian buttermilk and from similar Armenian products. They found that all the organisms investigated corresponded closely to the general characteristics as given by Löhnis for *Bacterium caucasicum*, Lehmann and Neumann (*Lactobacillus caucasicus* Kerne, Beijernick).

Morphologically they were long, rather slender bacilli, measuring about 1 $\mu$ in width by 2 to 50 $\mu$ in length, often growing in long chains and showing great variations in shape. The viable organisms stained by Gram, while the dead and involution forms were Gram-negative. Cultures were best obtained on media containing whey, malt, or milk. Colonies usually grayish-white, curled, filamentous, measuring 0.5 to 1 mm., rarely smooth and even. Coagulation of milk most rapid at 44° C. without peptonization.

In this group White and Avery were able to differentiate two types.

Type A shows organisms which stain homogeneously with Loeffler's methylene-blue or Neisser's stain, produce 2.7 to 3.7 per cent. lactic acid in milk, this lactic acid being always the inactive modification.

Type B shows organisms which exhibit intensely staining granules with Loeffler's methylene-blue or Neisser's stain, produce 1.2 to 1.6 per cent. lactic acid in milk, this acid being always the levorotatory modification.

The authors regard Type A as the true type of the Bulgarian bacilli and Type B as a paratype. It may be noted also that White and Avery were unable to obtain any constant result with carbohydrates other than dextrose and lactose, and that their Type A included in general various strains isolated from Bulgarian buttermilk, while Type B, the paratype, included organisms from Armenian milk products as well as an organism obtained from Kuntze and marked "Kornchenbacillus."

Finally Rahe[6] found that *Bacillus Bulgaricus* had no action on maltose and that it could be differentiated from the lactic acid organisms found in the intestinal contents. (See *Bacillus acidophilus*.)

1. Cohendy: Compt. rend. Soc. de biol., 1906.
2. Bertrand and Weissweiller: Ann. de l'Inst. Pasteur., 1906, xx, 977.
3. Bertrand and Duchacek: Ann. de l'Inst. Pasteur., 1909, xxiii, 402.
4. Heineman and Hefferan: J. Infect. Dis., 1909, vi, 304.
5. White and Avery: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1910, xxv, 161.
6. Rahe: J. Infect. Dis., 1914, xv, 141.

### Streptobacillus C of Grigoroff

Isolated from Bulgarian buttermilk by Grigoroff.

Short rods in chains of four, five, or six elements, at times fifteen to twenty. Gram-positive. Aërobe and facultative anaërobe. No growth on ordinary media. Good growth on sugar media except on lactose gelatin. Turbidity and whitish sediment in glucose broth with an acid reaction. Lactose broth also fermented. Milk coagulated by lactic acid. No coagulating ferment (rennin). No casease. True denitrifier. No indol. Produces buttermilk in twelve to fourteen hours at 37° C. Coagulates milk in six hours at 45° C. At 50° C. it coagulates milk in ten to twelve hours. Ferments saccharose without inverting it and ferments glycerin and levulose. No action on mannite, maltose, rhamnose, dulcite, or sorbite. Produces inactive lactic acid and a trace of alcohol (as shown by the iodoform test). Destroyed at 60° C. in seven to eight hours. Killed by 70° C. in one hour.

With *Bacillus* A and *Micrococcus* B produces true Bulgarian buttermilk.

Grigoroff: Rev. méd. de la Suisse Rom., 1905, xxv, 714.

### Bacillus sardous Grixoni

Found by Grixoni in a fermented milk product from Sardinia known as Gioddu.

Appears in milk in pairs or chains of six to eight elements. Stained with Loeffler's methylene-blue intensely staining granules appear near the poles of the orgnism. In old cultures the organisms are pale and the granules more intense. *Bacillus sardous* was not cultivated in pure culture on any media tried. Grows on gelatin plates with a characteristic Gioddu yeast, *Saccharomyces sardous*, as fine colonies with many fine sinuous prolongations in the vicinity of the yeast colonies. Such colonies are made up of streptobacilli. Grows also in broth with the yeast and here it exhibits motility.

Only *Bacillus sardous* and *Saccharomyces sardous* found by Grixoni in Gioddu.

Grixoni: Ann. di. med. nav., 1905, ii, Fasc. 3. Cited by Bertarelli. Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xv, 750.

### Bacterium mazun Weigmann, Gruber, and Huss

First observed by Düggeli[1] and cultivated by Weigmann, Gruber, and Huss.[2]

**Morphology.**—Long rods measuring 1 to 1.1 by 2.7 to 21 $\mu$ in milk, usually 1 to 9 $\mu$ in length. No branching. Usually in single elements, sometimes in twos, threes, and fours. Few organisms with bone-like protuberances on the ends. Protoplasm homogeneous. In whey agar stabs at 20° to 37° C. instead of the long rods, the cells vary greatly in length and breadth and show bladder-like swellings at the ends or along the rods. Like "involution" forms. Also

cells with true branching occur. The organisms vary in breadth from 0.6 to 1.75 μ. Long threads not uncommon, as, for example, a thread 0.6 μ broad and 210 μ long. One whey agar slants organisms showing true dichotomous branching are common, but side branching also occurs. Here the bacterial protoplasm is homogeneous. In older cultures where the acid has acted on the cells the protoplasm is vacuolated. In young whey cultures the organisms show regular morphology. In old cultures, three weeks, the cells are very irregular, showing swollen bladder-like masses in various areas and branching of different types. One end of the rod may be club-shaped, the other terminate in a fine thread. Often both ends are club-shaped. The irregularities in morphology appear in both stained and unstained preparations. Similar irregularities in peptone whey agar stabs and in peptone whey agar shake cultures. Non-motile.

**Stains** easily with carbolfuchsin. Gram-positive.

**Cultural Characters.**—Grows as an aërobe and facultative anaërobe at 20° and 37° C. Does not grow on ordinary gelatin, whey gelatin, lactose gelatin, dextrose gelatin, plain agar, lactose agar, broth, dextrose or lactose broth, or potato.

*Whey Agar.*—Superficial colonies in twenty-four hours small white points about 1 mm. in diameter in several days. They are flat, spreading, round or irregular, look like fat, and have the consistency of soft butter. Deep colonies white points becoming flat as they get older. The surrounding medium becomes turbid. Under low power (magnification ninety times) the superficial colonies are yellowish, round or irregular, more or less lobulated, coarsely granular, or striated. The edges are made up of fine hairy processes, later forming tangled skeins. The deep colonies are colorless or yellow, with brownish-red or rose-red centers. They look like a bunch of roots with numerous branches.

*Whey Agar Stab* at 20° or at 37° C.—Abundant growth along line of inoculation looking like a fir-tree after several days. The adjacent medium is acid and cloudy. At the end of about a month both at 20° and 37° C. the cultures look alike. Slight veil-like surface expansion.

*Whey Agar Shake Culture.*—At 37° C. in twenty-four hours the agar is permeated with fine points which in a few days appear as yellowish-white spheres of various sizes. These spheres may be provided with fine processes. About the colonies the agar becomes turbid. At 20° C. the development of the colonies is slower, but the appearance is the same.

*Whey Agar Slant.*—Grayish-white, thin, veil-like expansion, with lobulated or indented edges at both 20° and 37° C.

*Whey.*—At 37° C. abundant turbidity in twenty-four hours, with flocculent sandy sediment yellowish-white in color. In forty-eight hours more turbidity and sediment, both increasing till the development of acid stops the growth. Slower development at 20° C., but with the same characteristics. Cultures in whey agar containing peptone just like those in whey agar.

*Milk.*—At 20° C. not changed in forty-eight hours, converted to a firm, hard clot in seventy-two hours with almost no serum. Taste slightly acid. At 37° C. converted in forty-eight hours to a soft clot which becomes firm in three days. Almost no serum. Taste intensely acid.

*Acid production* tested in 500 c.c. flasks. Acidity at its maximum at 37° C. at the end of eighteen hours, 25 c.c. requiring 10 c.c. n/4 NaOH for neutralization. A second test at higher temperatures gave maximum acidity at 40° C., 25 c.c. requiring 18.1 c.c. n/4 NaOH for neutralization. Optimum temperature for acid production about 40° C.

1. Düggeli: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xv, 577.
2. Weigmann, Gruber, and Huss: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1907, xix, 70.

### The Körnchenbacillus of Luerssen and Kuhn

Found by Luerssen and Kuhn in commercial Bulgarian buttermilk together with *Bacillus bulgaricus*. A non-motile long slender Gram-positive bacillus tending to grow out into threads and chains.

With Neisser's stain and with alkaline methylene-blue the organisms occasionally show a peculiar granular staining when the growth is poor. About a third or half of the elements exhibit this appearance. When grown on unfavorable media it forms club-shaped, spiral, and curled forms. No growth at 20° C. Good growth at 37° to 45° C. Sparse growth at 50° C. Aërobic and facultative anaërobe. Slow scanty development on artificial media. Grows best on sugar media and in milk, occasionally in fluid media. Colonies on surface loose, curly, transparent, flocculent, ½ to 2 mm. in diameter, finer and smaller than anthrax colonies. Deep colonies curling striated skeins. White flocculent masses in fluid media.

Milk coagulated firmly and rapidly at 37° to 40° C. Reaction acid. No gas. No evident peptonization. The whey gives the iodoform reaction in twenty-four hours, but alcohol and related substances are present only in traces. The acid produced is the left polarizing

lactic acid. No growth on plain agar, potato, Endo agar, peptone solution, litmus whey, or ordinary broth.

This organism differs from *Bacillus bulgaricus* chiefly in its ability to grow well at 37° to 40° C. and in the firm clotting of milk as contrasted with the soft clot produced by the latter species. The presence of granules when stained by methylene-blue and Neisser's stain cannot be regarded as differential criteria.

Luerssen and Kuhn: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1908, xx, 234.

### Diplococcus lebensis Rist and Khoury

Described originally by Rist and Khoury from leben.

**Morphology.**—Appears always in pairs. Chains composed of diplococci, often in stage of division, proximate surfaces flat like the gonococcus. Fairly large, about $\frac{1}{3}$ $\mu$ in diameter. In the Leben it appears always as a diplococcus. In cultures it appears in short chains. In whey very long chains occur, composed of more than 100 individuals. No spores.

**Staining Reactions.**—Stains with the usual dyes. Takes on a bluish-black color with Gram's stain which it retains when young. Old organisms decolorize.

Vitality considerable. Cultures in milk last several months.

**Cultural Characters.**—*Lactose Agar.*—Colonies appear in twenty-four hours in the form of small drops, somewhat flattened, dirty-white, and translucent, with irregular borders. Colonies vary in size in the same tubes. Under low power they appear as yellowish disks with villous surfaces and are not characteristic. Older colonies darker, almost brown, with punched-out contours, almost like sponges.

*Deep Glucose Agar.*—Small white, spherical, opaque colonies with smooth surfaces.

*Lactose Gelatin Stab.*—Poor growth. Along line of inoculation a row of closely separated colonies, very small, round, white by reflected light, dark brown by transmitted light, with smooth edges. No liquefaction.

*Glucose Broth.*—Abundant rapid development, with the production of a turbidity and a coherent grayish-white granular precipitate.

*Whey.*—Excellent growth with the production of volatile substances with the odor of cheese and lactic acid. This last amounts to 0.396 per cent. in twenty-four hours.

*Milk.*—Rapid coagulation. In milk with sodium carbonate to neutralize the acidity coagulation also occurs, so that the organism produces a rennin-like ferment.

Rist and Khoury: Ann. de l'Inst. Pasteur, 1902, xvi, 65.

### Micrococcus B of Grigoroff

Obtained from Bulgarian buttermilk by Grigoroff.

**Morphology.**—Cocci in single elements, diplococci, or in groups.

**Staining Reactions.**—Gram-positive.

**Cultural Characters.**—Aërobe and facultative anaërobe. Growth poor on ordinary media or on sugar media. Turbidity in plain or sugar broth with a deposit along the sides and on the bottom of the tube. Reaction acid. Lactose broth also fermented. Opaque whitish colonies on gelatin. On gelatin slants a transparent whitish expansion with a slow liquefaction at the end of about a week. Liquefaction never complete. Gelatin stab shows colonies along line of inoculation with a nail-head surface expansion. True denitrifier. Gives nitrose-indol reaction. Milk coagulated by lactic acid, not by ferment action. Coagulum partially dissolved by casease.

Produces a buttermilk like the Bulgarian product in a little less than twenty-four hours at 37° C. At 45° C. coagulates milk in seven to eight hours. It resists 50° C., but coagulates more slowly in eighteen to twenty-four hours. Killed at 60° C. in an hour.

Ferments mannite and saccharose without inverting the latter. Ferments also glycerin, maltose, rhamnose, and levulose. No action on dulcite and sorbite. Produces inactive lactic acid and a trace of alcohol as shown by the iodoform test. With *Bacillus* A and *Streptobacillus* C it produces true Bulgarian buttermilk.

Grigoroff: Rev. méd. de la Suisse Rom., 1905, xxv, 714.

### Streptothrix from Dadhi

Described by Chatterjee from Dadhi, the fermented milk of India.

**Morphology.**—In the fermented milk parallel bacilli with square-cut ends, 7 to 8 $\mu$ in length and 2 $\mu$ in breadth. Stained by methylene-blue the organisms show pink granules on a blue background. Granules not stained by Leishman, thionin blue, or Neisser. Best seen

in twenty-four-hour cultures and vary in number from two to ten. When only two are present they are terminal. Gram-positive, non-motile. No spores.

**Growth** only on glucose or lactose agar with streptococcus-like colonies. Growth along line of inoculation in glucose agar with clouding of the medium. In cultures on *glucose agar* the organism appears as long thick filaments without granules, convoluted and twisted, corkscrew, 40 to 50 $\mu$ long, with twenty to thirty turns in the screw. The granules appear, however, in milk cultures from the glucose agar tubes. *Milk* curdled in twelve hours with a solid coagulum and large amounts of lactic acid. No whey. Organisms survive in such milk only seven days. Coagulation due to enzyme activity as well as to lactic acid. Milk sugar converted to lactic acid. No action on fatty constituents of the milk. No butyric acid.

No indol. No gas formation.

In mixed cultures with *Bacillus typhosus*, *Bacillus coli*, Gärtner, *Paratyphoid* B, *Vibrio comma*, dysentery bacilli, diphtheria bacilli, all these organisms are killed in forty-eight to seventy-two hours.

Like the Körnchenbacilli of Lehmann and Neumann, "Bulgaricus," "lebensis," etc. Regarded by author as streptothrix because of convoluted twisted chains and granules. No evidence of branching, hence cannot be a true streptothrix.

Chatterjee: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1910, liii, 103.

### LACTOBACILLUS BOAS-OPPLERI (Boas and Oppler)

This organism, known usually as the Oppler-Boas bacillus, was reported from the medical clinic of Dr. Boas in Berlin by Oppler,[1] having been found in the stomach contents of patients with gastric cancer. It appeared as a fairly slender rod growing out into long threads and often arranged in zigzags, and was characterized by its retention of Gram's stain. While not cultivated by Oppler, it was thought to be the cause of the lactic acid which appears in the stomach contents of patients with gastric carcinoma, and that its recognition might be of help in the diagnosis of this disease. Ever since this early observation the finding of long Gram-positive bacteria in the stomach has been much discussed and opinions have differed as to the significance of the observation. Thus Strauss[2] found similar organisms in the normal gastric fluid. Smears from the stomach showed bacteria staining solidly with carbolfuchsin and giving granules when stained with methylene-blue. The granules were quite large and separated by unstained areas which looked like spores. The organisms retained Gram's stain. Strauss cultivated this organism in agar to which gastric juice was added, where it produced leafy colonies, and in milk which was coagulated in three days. In the same year Schlesinger and Kaufman[3] found similar organisms in 19 out of 20 cases of gastric carcinoma and succeeded in cultivating them on meat infusion peptone agar with glucose added and in beerwort. Sick,[4] however, again showed that Gram-positive bacteria are present in the stomach contents of normal individuals. Finally, Rodella[5] came to the conclusion that the Oppler-Boas bacillus is identical with *Bacillus acidophilus* of Moro and *Bacillus bifidus* of Tissier, while Heineman and Hefferan[6] identified it with the various types of Bulgarian bacilli. In the light of our present knowledge neither of these conclusions was justified. For the present the Oppler-Boas bacillus should be regarded as a parasitic lactic acid bacillus present probably in both normal and pathological cases and possibly identical with similar lactic acid organisms of the saliva. It has not thus far been identified definitely with any of the environmental lactic acid organisms. From various observations, especially those of Ficker,[7] who found it in nearly all of 148 cases examined, this organism is probably universally present in the stomach of pathological cases where the normal hydrochloric acid is diminished or absent and does not develop in normal individuals, possibly being inhibited by the acid present. It should be pointed out that this conclusion is not strictly in harmony with the observations on the cultivation of the organism, since it grows well in acid media.

**Morphology.**—The Oppler-Boas bacillus appears in the stomach contents and in artificial cultures as a long non-motile rod, from 2 to 10 $\mu$ in length and about 1 $\mu$ in width, often growing out into long threads. It retains Gram's stain and stains irregularly with methylene-blue, showing blue and red metachromatic granules. Ficker has found the granular appearance frequently in smears of the organism from the esophagus and suggests that they are due to starch. According to Rodella the Oppler-Boas bacillus in acetic acid broth shows club-shaped forms with clubs at one or both ends, long threads, and true branching in both fluid and solid media. The terminal swellings often form an angle with the rods like a crutch. In cultures under anaërobic conditions many bizarre forms may be found, some divided at both ends and looking like an X. When stained with methylene-blue the organisms showed protoplasmic tags which tended to be extruded from the bodies of the cells. Rodella's observations have not been confirmed and his descriptions suggest that his cultures of the Oppler-Boas bacillus contained either *Bacillus acidophilus* or *Bacillus bifidus*.

**Methods of Cultivation.**—The Oppler-Boas bacillus can be cultivated in fluid media to which extracts of gastric cancer are added and in both fluid and solid media containing beer-wort or glucose. Cultures are best obtained originally by making inoculations into fluid media and transferring to dextrose agar. On solid media the colonies are small and round with irregular edges. It grows both aërobically and anaërobically and especially well in mixed cultures on plates. Optimum temperature 37° to 41° C.

According to Sick this species ferments glucose, levulose, saccharose, lactose, and maltose, not glycerin or the polysaccharids. He states that the organism produces lactic acid only in traces, but much volatile fatty acid. The addition of cell extracts, such as the thymus, to cultures enables the organisms to manufacture large quantities of lactic acid. Rodella suggests that this is not to be traced to the nitrogen, but to some unknown influence of cell extracts on the metabolism of the organism. This reaction would thus be analogous to the favorable influence of cell extracts on the growth of anaërobes.

1. Oppler: Deutsche med. Wchnschr., 1895, xxi, 73.
2. Strauss: Ztschr. f. klin. Med., 1895, xxviii, 578.
3. Schlesinger and Kaufman: Wien. klin. Rundschau, 1895, ix, 225.
4. Sick: Deutsch. Arch. f. klin. Med., 1906, lxxxvi, 370.
5. Rodella: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1908, xlvii, 445.
6. Heineman and Hefferan: J. Infect. Dis., 1909. vi, 304.
7. Ficker: Arch. f. Verdauungskr., 1908, xiv, 537.

### BACILLUS ACIDOPHILUS Moro

Described by Moro[1] from the dejecta of infants. Isolated independently by Finkelstein[2] and reported in the same year. Investigated with especial care by Tissier.[3]

**Historical.**—Moro states that Escherich obtained in 1898 branching organisms from stools showing Gram-positive rods. Similar organisms were found in the urine in purulent cystitis. Gram-positive organisms were isolated by Moro from the stools of breast-fed infants by the use of acid beerwort broth. The sediment was transferred to acid beerwort agar on which fine irregular radiating colonies with branched edges developed. Superficial colonies appeared only on anaërobic plates and were made up of thick skeins of long twisted threads. Growth best at 37° C., also at 20° to 22° C. Both aërobic and anaërobic development. Produced a flocculent sediment in broth. Growth sparse in milk. Acid production was moderate at first in the bottom of the tubes, but increased slowly with an eventual coagulation. Found in stools of milk-fed infants and in older children. No branching . No spores.

This organism was clearly differentiated by Moro from *Bacillus bifidus* of Tissier, which shows branching and club-shaped forms in the stools and branching, club-shaped forms and elements like candle flames in shape in cultures. *Bifidus* also grows only anaërobically and takes Gram's stain badly.

Similar organisms were isolated by Finkelstein by putting the stool material in 0.5 to 1 per cent. acetic acid broth with 2 per cent. glucose. After twenty-four to forty-eight hours the material was transferred to sugar agar. Finkelstein found that peculiar branching organisms producing threads were constantly present in the stools of breast-fed and bottle-fed children. These he regarded as identical with *Bacillus acidophilus* of Moro, but expressed the opinion that there were probably three or four other species. The organisms were found in both normal and pathological cases, but had no definite pathological properties.

In view of our present knowledge it seems possible that the branching organisms observed by Finkelstein may have been *Bacillus bifidus* of Tissier and not *Bacillus acidophilus* of Moro. The difficulty of separating these species is well illustrated by the work of Rodella,[4] who came to the conclusion that the Oppler-Boas bacillus found in the stomach in gastric carcinoma, *Bacillus bifidus* of Tissier, and *Bacillus acidophilus* were all the same species.

The following description is taken from Tissier, who isolated this species in deep glucose agar tubes of Veillon. The organisms were found in the stools of children fed on a mixed diet, on ordinary or sterilized milk, and also in cases of diarrhea. They were not found by Tissier in breast-fed children.

Fig. 109.—*Bacillus acidophilus* from milk culture stained with gentian-violet.

**Morphology.**—Large stubby bacillus with round ends varying greatly in length. The elements may be short, 4 to 5 $\mu$ long, or several times as long. Usually rigid, rarely bent. Very regular. Distributed uniformly in films. On aërobic media it appears as a short coccobacillus like *Bacillus coli*, but longer and thicker. Usually in twos and threes. In liquid media it appears in short chains, two to four elements in each. In anaërobic cultures both short forms and long sinuous forms appear. After several transfers on anaërobic media the long forms predominate, straight, slightly curved, or undulating. The ends of the usual rods are often shaped like a spatula, swollen as if spores were being formed. The long undulating forms usually have pointed ends. No branching. In mixed cultures with streptococci or *Bacillus exilis* the rods are usually short, slightly curved, or twisted. Non-motile. No spores.

Vitality considerable. Transfers successful after two months. Destroyed at 80° C. in fifteen minutes.

**Staining Reactions.**—Stains with the basic anilin dyes, the filaments often showing unstained areas. Stains uniformly by Gram's method.

**Cultural Characters.**—*Bacillus acidophilus* grows at 20° and at 37° C. It is not a pure aërobe, since it will not develop well on aërobic media. Nor is it a strict anaërobe, since it does not grow well in the depths of media.

*Plain Agar Slant.*—Colonies appear in about fifty-eight hours as fine transparent points which become whitish and a trifle more opaque. Under the microscope the colonies show acuminated centers surrounded by granular borders. Water of condensation turbid.

*Wertheim's Agar.*—Colonies appear in forty-eight hours and reach their maximum development in five to six days. They show numerous ramifications projecting into the agar.

*Glucose Agar Slant.*—More abundant development, colonies larger and whiter. Water of condensation turbid.

*Deep Glucose Agar.*—Characteristic colonies develop in twenty-four hours, about 5 cm. below the surface. The isolated colonies gradually increase in size till they attain a diameter of about 3 mm. Where the colonies are thicker in the middle of the tube they remain smaller, and in the aërobic zone near the surface they retain their small size. Colonies are irregularly

lobulated, with multiple hairy prolongations resembling a mass of hair or a tuft of moss. Yellowish-gray in color. No gas.

Growth similar in *deep plain agar*, in *deep acid agar*, but not so good as in *glucose agar*.

Growth poor in *plain* or *glucose broth*. No turbidity. Insignificant sediment arising as a spiral on shaking.

*Milk*.—Fair growth. The milk becomes acid in five to six days and coagulates in ten to fifteen days. At the end of two months it is acid and the clot not retracted.

No growth on *plain* or *glycerin potato* or *glucose gelatin slants*. In *deep glucose gelatin* fine irregular embossed colonies appear in about five to six days.

Some strains are more anaërobic than others, growing in the depths of agar and giving a ring below the aërobic zone. Many strains can be accustomed to laboratory conditions growing well on ordinary agar. Other strains preserve their original character.

Non-pathogenic for mice and guinea-pigs.

1. Moro: Wien. klin. Wchnschr., 1900, xiii, 114.
2. Finkelstein: Deutsche med. Wchnschr., 1900, xxii, 263.
3. Tissier: Recherches sur la flore intestinale des Nourisson. Paris, 1900.
4. Rodella: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1908, xlvii, 445.

**Varieties of Bacillus Acidophilus.**—In a sweeping and painstaking investigation of the organisms growing in acid media Mereschewsky[1] and his pupils have brought out many new facts in regard to this group. By planting fecal material in 0.5 to 1 per cent. acetic or lactic acid broth they have cultivated acidophilic or aciduric organisms from a variety of animals, in fact, from practically every species examined and from man in nearly every condition. These organisms they differentiate into two types by the appearance of the colonies.

Type I produces small colonies the size of a needle head, round or oval, white and opaque. In the immediate vicinity the agar becomes turbid and the colonies seem to be surrounded with aureoles. Under low power the colonies are round or egg-shaped, dark brown, opaque, surrounded by indistinct aureoles which are often spread along the sides of the oval colonies.

Type II produces smaller colonies which are distinctly differentiated from the agar and show no aureole formation. As the colonies mature they produce extremely fine short tooth-like extensions or outgrowths. Under low power the colonies seem to be made up of brown root-like masses sending out branches in every direction.

According to Bjeloussow[2] Type I, which he calls *Bacillus acidophilus* No. 1, is the same as *Bacillus bifidus* of Tissier, and Type II, which he calls *Bacillus acidophilus* No. 2, is the same as *Bacillus acidophilis* of Moro. He was unable to differentiate the two organisms by their cultural reactions, although *Bacillus acidophilus* No. 1 was much more resistant to the action of acid than *Bacillus acidophilus* No. 2. Both types came from Gram-positive organisms which never showed true branching. In the light of the very accurate work of Tissier the identification of this Type I with *Bacillus bifidus* is not justified.

More recently Rahe[3] has investigated carefully the acid-resisting organisms in this group. He finds three types of bacteria.

*Variety A* produces only the Mereschewsky Type II colony, coagulates milk in twenty-four hours at 37° C. with a large amount of lactic acid, does not ferment maltose, and has no constant fermentation of saccharose. This type Rahe regards as the true *Bacillus bulgaricus*. It is a milk organism "sui generis," appearing in the intestine only after ingestion and surviving there only when ingested in enormous numbers. Morphologically it appears as a long Gram-positive bacillus showing buds and stems, as mentioned by White and Avery, as well as threads and branches. It produces an acidity varying from 0.9 to 2.3 per cent. estimated as fixed acid in terms of lactic acid.

*Variety B* produces both the Type I and Type II Mereschewsky colonies, splits maltose actively, saccharose occasionally, and coagulates milk in two to six days, usually in three to four. It produces an acidity varying from 0.2 to 1.3 per cent. estimated as fixed acid in terms of lactic acid, this being about half the amount produced by Variety A. Morphologically it is a long or short streptobacillus showing bipolar staining with Loeffler's dye.

*Variety C* ferments maltose, but does not coagulate milk, producing only 2 to 3 per cent. normal acid or less than 0.2 per cent. estimated as fixed acid in terms of lactic acid. This variety also produces both types of Mereschewsky colonies. Morphologically it appears either as a long bacillus or as a streptobacillus.

While Rahe regards his Variety A, which has no action on maltose, as the true Bulgarian bacillus, he considers Variety B and Variety C as essentially fecal bacteria. His Variety B seems to correspond closely to the true *Bacillus acidophilus* of Moro, and his *Variety C* to the organism originally described by Finkelstein, which, according to Lehmann and Neumann, does not coagulate milk.

1. Mereschewsky: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1905, xxxix, 380, etc.
2. Bjeloussow: Zur Biologie und methodik der Ausscheidung der sogenannten acidophilen Bakterien. Diss. No. 76, St. Petersburg.
3. Rahe: J. Infect. Dis., 1914, xv, 141.

### BACILLUS EXILIS Tissier

First isolated from the stools of infants and described by Tissier. Obtained by the use of Veillon tubes of deep glucose agar.

**Morphology.**—Appears in the stools and in liquid media as fine rigid rods, in single elements, pairs, and short chains. Often slightly bent and united alternately end to end, this giving a flexuous appearance to the chains. Long forms appear sparsely in old cultures, regular ends, square, slightly curved. Less polymorphic than *Bacillus acidophilus*. Nonmotile. No spores.

**Staining Reactions.**—Stains well with dilute fuchsin, gentian-violet, and thionin. Retains Gram stain.

Vitality considerable, transfers after ten to fifteen days being successful.

**Cultural Characters.**—Grows well at 20° to 37° C. aërobically and anaërobically on glucose media, but not on acid media.

*Agar Slant.*—Water of condensation turbid in twenty-four hours. At the end of forty-eight hours fine, bluish colonies appear on the surface. Colonies increase in size, become more transparent and white, then decolorize, and become invisible in old cultures. They are never larger than needle points. Under low power they seem to be uniformly thick with round edges.

*Glucose Agar.*—Development more rapid. Colonies appear in twenty-four hours and are about ½ mm. in size in forty-eight hours.

*Broth.*—Whitish filamentous deposit.

*Milk.*—Coagulation in eight to ten days. Coagulum soft and does not retract.

No growth in plain gelatin, glucose gelatin, plain potato, or glycerin potato.

*Deep Glucose Agar.*—Growth good. In twenty-four hours small regular oval colonies with entire edges throughout the agar. No gas.

*Non-pathogenic.*

Tissier: l. c., page 102.

### BACILLUS ACIDOPHIL-AËROGENES Torrey and Rahe

Obtained by Torrey and Rahe from human dejecta in 7 adults and 2 infants in an examination of 37 individuals. Found only in one instance in 24 examinations of very young infants. Believed by the authors to be present universally in the intestinal tract of most individuals, but in much smaller numbers than the non-gas-producing *Bacillus acidophilus* of Moro. Also isolated from the feces of sheep and hens, but not from dogs, monkeys, rabbits, guinea-pigs, white rats, white mice, geese, and pigeons. Probably widely distributed in nature.

**Morphology.**—In unneutralized glucose broth twenty-four hours old the organisms vary in length from 1.5 to 11.5 $\mu$ and are about .8 $\mu$ in width. The majority are about 5 $\mu$ in length, but strings 40 $\mu$ in length are nearly always present. On glycerin agar the organisms are thicker and longer than when grown in sugar broth or in oleate agar. Occasionally branching forms or Y forms are present and strings are also frequently present, long and curved. Nonmotile.

**Staining Reactions.**—Uniform staining by Loeffler's alkaline methylene-blue. Strongly Gram-positive.

**Methods of Isolation.**—Easily isolated from the dejecta by adding a small amount of material to +5 acetic acid glucose broth in fermentation tubes and incubating from two to three days. The organism is present in the tubes which show fermentation when this is not due to yeast as indicated by the pellicle formation. From the positive fermentation tubes the material is streaked on glucose oleate agar, from this transferred again to acetic acid broth, incubated for two days, and finally transferred to glucose broth tubes with the natural acidity from the meat not neutralized.

**Cultural Characters.**—Growth at 37° C. No growth at room temperature.

*Glucose Oleate Agar Plates.*—Colonies may be small, round, white, opaque, about 1.2 mm. in diameter (Type I of Mereschewsky) or gray semitransparent, smaller than a pin-head in size with short tooth-shaped projections (Type II of Mereschewsky).

*Glycerin Agar* (+1 in reaction).—Streptococcus-like colonies appearing on the second day.

*Sugar-free Agar* (+1 in reaction).—Streptococcus-like colonies.

*Sugar Broth.*—Growth with a marked tendency to adhere to the bottom and the sides of the tube. On shaking, a heavy turbidity like that of *Bacillus Bulgaricus*.

*Milk.*—Growth in milk poor or absent. In one strain milk was clotted in seventy-two hours, while in thirteen other strains no clotting occurred after fifteen to twenty days' cultiva-

tion. In six cultures there was no apparent effect. The clot when formed was soft and separated slightly from the whey. No gas from milk cultures.

*Fermentation tubes* made from unneutralized sugar-free broth by adding 2 per cent. of the sugars.

Active fermentation with gas and acid from dextrose, maltose, saccharose, lactose, and raffinose at 37° C. No growth in mannite or dextrin broth.

Acid formation from sugar media more active than with *Bacillus Bulgaricus* or *Bacillus acidophilus*. At the end of three days usually 10 per cent. normal acid produced by this organism from unneutralized dextrose broth as compared with 5.61 per cent. for *Bacillus Bulgaricus* in five days, 5.66 for *Bacillus acidophilus* Group II in five days, and 5.09 for *Bacillus acidophilus* Group III in five days.

Maximum gas production at the end of seventy-two hours 60 per cent., and minimum 2 per cent., average amount for dextrose 23 per cent. Gas predominantly hydrogen, the formulas varying from

$$\frac{H}{CO_2} = \frac{4}{1} \text{ to } \frac{H}{CO_2} = \frac{6}{1}$$

*Rate of Growth.*—Growth rapid in unneutralized glucose broth, a distinct cloud appearing in seven hours, at which time *Bacillus Bulgaricus* and *Bacillus acidophilus* show no growth.

*Survival.*—These organisms die out rapidly at 37° C. because of the high acidity. In one strain the organism survived eight days at 37° C. in glucose broth, white *Bacillus Bulgaricus* survived six days and *Bacillus acidophilus* survived fifteen days. At room temperature *Bacillus acidophil-aërogenes* died out in fifteen days and *Bacillus Bulgaricus* in six days. At the temperature of the ice-box all three organisms were alive at the end of fifty days.

**Animal Reactions.**—Rabbits inoculated with material from glycerin agar cultures after three or four injections produced agglutins of a strength as great as 1 to 800. In 14 strains 10 reacted positively to a serum made by one of the strains. Strains of *Bacillus acidophilus* of Moro did not react. The agglutinating serum made from a strain of *Bacillus acidophilus* of Moro had no action upon strains of *Bacillus acidophil-aërogenes*. The organisms in this group of *Bacillus acidophil-aërogenes* are, therefore, apparently more homogeneous than the organisms in the group represented by *Bacillus acidophilus* of Moro.

Torrey and Rahe: J. Infect. Dis., 1915, xvii, 437.

### Bacillus bifidus communis Tissier

Described first by Tissier in 1899 and subsequently isolated repeatedly from the stools of infants and described at length. Clearly differentiated from *Bacillus ramosus* and *Bacillus furcosus* of Veillon and Zuber.

*Distribution.*—According to Tissier this organism forms the principal ingredient of the stools of breast-fed infants. It is also present in stools of bottle-fed infants and can be obtained in cases of diarrhea, but with difficulty, because of the preponderance of fermenting bacteria.

**Morphology.**—In the stools it appears as a slender bacillus with pointed ends varying in length from 2 to 3 to 5 to 6 $\mu$, with an average length of 4 $\mu$. It is usually arranged as a diplobacillus with the proximal ends swollen and the distal ends tapering or pointed, separated by unstained areas. These diplobacilli usually lie side by side in parallel bands. In cases of diarrhea longer irregularly swollen forms are present. Many of the organisms show true branching as Y-shaped forms. The branches may be the same diameter or may terminate in swollen extremities. In young cultures the organisms have much the same appearance as in the stools. They appear as diplobacilli with pointed ends. In older cultures the organisms are elongated with club-shaped ends, geniculated, joined to each other by pointed extremities with a central swollen mass. Here many of the organisms show true branching and are arranged with great irregularity. When the cultures are still older the organisms are much elongated and more show true branching, which may occur at one end or at both ends. The ends of the branches may be pointed or club-shaped. Sometimes a third branch may occur between two others, giving an appearance like a trident. Vesiculated or bladder-shaped forms are also common, staining only at the poles, together with racket-shaped forms. These swollen and elongated forms appear chiefly in old acid media. In rapidly growing stages the organisms remain short for the most part. Grown with other bacteria, *Bacillus bifidus* undergoes many modifications. It may present short bifurcated organisms or irregularly swollen vesiculated elements, interspersed with thin irregularly branching Y-shaped forms. According to Tissier the elongated and bifurcated forms depend upon the reaction of the media. The vesiculated bodies represent dead organisms.

Non-motile. No spores.

**Staining Reactions.**—Young elements stain uniformly with basic anilin dyes. In stools and young cultures the central part stains well, the peripheral portions lightly. In old organisms the cytoplasm does not take the stain, while the central areas, the extremities, and the points of bifurcation stain well. The vesiculated elements stain badly, only the shrunken points taking up the dye. In pure cultures one can find every variation from unstained to deeply stained bacilli. Young elements retain Gram's stain uniformly. Older forms partially decolorize. With Gram's stain and a red counterstain like alcoholic fuchsin all shades of color are presented—red, partially red, partially blue, and blue. This chromophil reaction, first described by Escherich in the stools, can be duplicated in pure cultures of this bacillus stained by Gram and a red counterstain.

Vitality considerable. Can be transferred successfully from cultures three months old.

**Cultural Characters.**—*Bacillus bifidus* is a strict anaërobe growing best at 37° C., but slowly at 22° C. It may be isolated best by the use of Veillon tubes of deep glucose agar.

Colonies in *deep glucose agar* at the end of three days are fine, regular, and ovoid, whitish in color, and appear in a zone about 3 cm. below the surface. Under the microscope the colonies are alike at this stage. At the end of five days some of the colonies are large and lenticular, while others are small and ovoid. In the depths of the tube some of the colonies may become much larger. The typical colonies are lenticular, regular with entire edges, often showing small projections. They may be 4 mm. in diameter, but average 2 mm. The typical form

Fig. 110.—*Bacillus bifidus* from milk cultures. Stained by Gram.

of the colony is lost when the organism grows with other species. The colonies may be much larger, 5 to 6 mm., as with *Coccobacillus perfœtens*, or embossed, as with *Streptococcus intestinalis* or *Bacillus acidophilus*. No gas, but acid from glucose.

No growth in *plain agar* at 22° or at 37° C. in sulphureted agar without sugar or in acid agar. In *glucose agar*, with one-twentieth sterile bile made alkaline, *Bacillus bifidus* produces fine slowly growing colonies. No growth in *deep gelatin* covered by a plug of agar. Growth abundant in *glucose broth* under strict anaërobic conditions. At the end of three days a turbidity appears with a flocculent easily-broken sediment. At the end of eight to ten days a thick, ropy, viscid sediment develops, while the broth does not again become limpid. Under anaërobic conditions *Bacillus bifidus* develops in milk without coagulating the casein. Surface growth in media practically never occurs because of its extreme sensitiveness to oxygen. Growth practically only in sugar media.

Agar cultures killed at 80° C., and then neutralized, give no growth on fresh inoculation. The nutritive material in the agar is exhausted.

*Bacillus bifidus* is non-pathogenic, pure cultures having no effect on white mice or guinea-pigs. It augments the virulence of such low virulent organisms as *Staphylococcus albus*.

Thus when *Staphylococcus albus* alone kills white mice in ten to fifteen days by subcutaneous inoculation, this organism plus *Bacillus bifidus* kills mice in three days.

Tissier: Recherches sur la flore intestinale des nourrisson. Paris, 1900, p. 85.

## Tribe 6. Pasteurelleæ Com.

Gram-negative rods, showing bipolar staining. Parasitic forms of slight fermentative power.

### GENUS 8. PASTEURELLA Trevisan

Aërobic and facultative. Powers of carbohydrate fermentation slight; no gas produced. Gelatin not liquefied. Parasitic, frequently pathogenic, producing plague in man and hemorrhagic septicemia in the lower animals. Gram-negative.

The pasteurella group, usually known as the hemorrhagic septicemia group, was established by Hueppe[1] in 1886, who recognized that the organisms of Perronciti[2] and Pasteur[3] in fowl cholera, of Kitt[4] in Bollinger's "Wild-und Rinderseuche," of Flügge in rabbit septicemia, and of Loeffler[5] and Schütz[6] in "Schweineseuche" were similar in morphology, cultural reactions, and pathogenic action on animals. Similar organisms were soon isolated from a variety of animal diseases by Oreste and Armanii[7] in "Buffelseuche," by Poels[8] and Hueppe in septic pleuropneumonia of cattle, by Galtier[9] in septicemia of sheep. The designation "pasteurella" proposed by Trevisan has now generally displaced Hueppe's term, especially in America. Representatives of this group are responsible for disease in practically every species of animal, but have been found rarely in man (except B. pestis). They cause the wide-spread septicemias so often seen under natural conditions and a great variety of other lesions. The virulence of the organisms varies greatly; strains from one animal frequently have little pathogenic action upon other species. The virulence is lost rapidly under artificial conditions. Organisms which have the characteristic morphology, cultural features, and pathogenic action of members of this group are found normally in healthy animals, in the intestines, in the nose and throat, in the tonsils, on the buccal mucosa, and in the saliva.

In the opinion of Hutyra[10] the only specific diseases in which these organisms are clearly established as the etiological agent are fowl cholera, Bollinger's Wild- und Rinderseuche, septic pleuropneumonia of calves, and swine plague (Schweineseuche).

All varieties of hemorrhagic septicemia are intensely virulent for mice and rabbits and somewhat less so for guinea-pigs. Depending upon the source, different strains exercise almost a selective action upon different species. Thus hens and pigeons are easily infected with the organisms from fowl cholera; with difficulty or not at all with strains from mammals. Sheep are easily infected with organisms from sheep septicemia, but have considerable resistance to "Wildseuche" and swine plague. A representative of the groups from ducks, the bacillus of "Entencholera" of Cornil and Toupet,[11] is easily transferred to waterfowl, but not to hens. There are many exceptions to such general rules, however. Thus the strains of swine plague isolated in America are regularly virulent to pigeons and some of the European strains also (Schütz). Affanassieff[12] reports that virulent swine plague material regularly kills pigeons and hens, while Voges produced fatal infections in hens by feeding swine plague organisms and in swine by the fowl cholera organisms. Gaffky[13] has shown that both pigeons and hens are sensitive to the virus of rabbit septicemia. Furthermore, strains isolated from individual species of animals vary greatly in virulence for this

species, producing lesions which vary from a rapidly fatal septicemia to mere local inflammatory changes which tend to become chronic. The virulence of the isolated strains is frequently lost in cultivation in the laboratory and weakly virulent strains can be made highly virulent by passage through appropriate animals.

The characteristic pathogenic action of all members of this group consists of a rapidly fatal bacteremia with hemorrhages and inflammatory edema at the site of inoculation and in different parts of the body. In rabbits after cutaneous or subcutaneous inoculation death follows in six to twenty-four hours from virulent cultures. At autopsy the animals show an acute swelling of the lymph-nodes and spleen, hyperemia and edema of the lungs, hemorrhagic tracheitis, hyperemia of the kidney, acute intestinal catarrh, punctiform hemorrhages in mucous membranes and air passages, intestinal mucosa and serous membranes. With subcutaneous injection there is extensive edema at the site of inoculation, the tissues being saturated with turbid serous fluid and showing minute hemorrhages. In pigeons and hens subcutaneous inoculation over the muscles of the breast produces a straw-yellow exudate which penetrates between the grayish discolored bands of muscles. With material of low virulence a caseous, dry sequester develops which frequently sloughs. The animal may recover or die of chronic cachexia.

In guinea-pigs the same type of infection develops as in rabbits. In large animals—horses, cattle, sheep, and swine—subcutaneous inoculation rarely leads to fatal septicemia. As a general rule, only a wide-spread inflammatory edema or purulent inflammation develops at the site of inoculation. This usually sloughs and heals.

With intravenous administration all species of animals may be infected. With virulent material the animals develop a hemorrhagic septicemia from which they may die in six to eight hours. With less virulent material they show a chronic disease with inflammatory exudates in various areas, especially the joints, are liable to secondary infection, and frequently die of cachexia.

Intraperitoneal and intrapleural inoculation is likewise followed by fatal infection, the serous membranes showing a serosanguineous exudate poor in cells.

Various species of small animals may be infected by feeding with material from infected animals or cultures. Thus feeding material from fowl cholera leads to infection of hens, geese, ducks, pigeons, and small birds, and occasionally to mice and rabbits. Similar material from mammals may usually be fed without producing infection. Mammals cannot usually be infected by feeding, even when the virus comes from a disease to which they are naturally sensitive. Guinea-pigs are usually resistant to feeding infection.

Organisms of this group possess a special affinity for the serous membranes and for lung tissue which may show an acute croupous or hemorrhagic-croupous inflammation. In chronic cases large necrotic foci of dry caseous material are seen.

The typical organisms are present in the blood in acute cases in great quantity. They are also present in the various organs and in the lesions of almost every type. They may be difficult to find in the caseous foci in the lungs in chronic cases, since this material often contains secondary invaders.

The members of this group possess an extremely high degree of virulence at times. Thus Davaine succeeded in infecting rabbits with 1/100,000,000

drop of the blood of an infected ox. Preisz produced a fatal infection in a rabbit with 5 oesa of a culture of swine-plague diluted a trillion times by rubbing it beneath the skin. Schonwerth[14] estimates that a single bacterial cell will produce infection in a susceptible animal. This may be due to a general failure of phagocytosis against the organisms of this group.

A case of human infection with an organism of the hemorrhagic septicemia group has recently been reported by Meyer and Höppli.[15]

Type species *Pasteurella cholerœ-gallinarum* (Flügge) Trevisan.

" *lepiseptica.*
" *suiseptica.*
" *boviseptica.*
" *vituliseptica.*

*Bacillus pestis* Kitasato and Yersin.
*Bacterium tularense* McCoy and Chapin.

1. Hueppe: Berl. klin. Wchnschr., 1886, xxiii, 753.
2. Perronciti: Arch. f. wissensch. u. prakt. Tierh., 1879, v, 22.
———: Rec. de méd. vét., 1880, p. 523.
3. Pasteur: Compt. rend. Acad. d. sc., 1880, xc, 239.
———: Rec. de méd. vét., 1880, 125, etc.
4. Kitt: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 1903, ii, 559.
5. Loeffler: Mitt. a. d. K. Gsndhtsamte, 1884, ii, 421.
———: Arb. a. d. K. Gsndhtsamte, 1886, i, 46.
6. Schütz: Arb. a. d. K. Gsndhtsamte, 1886, i, 376.
7. Oreste and Armanni: Atti r. Ist. d'Incorag. a. sc. nat. di. Napoli, 1887, vi.
8. Poels: Fortschr. d. Med., 1886, iv, 388.
9. Galtier: Bull. Soc. vét., 1891, p. 97, 208.
10. Hutyra: Septicæmia hæmorrhagica. *In* Handb. d. path. Mikroörg. (Kolle and Wassermann).
11. Cornil and Toupet: Bull. de la Soc. Nat. d'aclimat., June 20, 1883.
12. Affanassieff: Arb. a. d. Geb. d. path. anat. Inst. zu Tübing., H. 2, 1892, i.
13. Gaffky: Mitt. a. d. K. Gsndhtsamte, 1881, i, 50.
14. Schonwerth: Arch. f. Hyg., 1892, xv, 61. *Ibid.*, 1893, xvii, 361.
15. Meyer and Höppli: Centralbl. f. Bakteriol. [etc.], 1923, xv, 241.

### PASTEURELLA CHOLERÆ-GALLINARUM (Flügge) Trevisan

The etiological agent of fowl-cholera, known also as *Bacillus bipolaris avisepticus*, *Bacterium avisepticum*, and *Bacterium avicidum*, was apparently first recognized by Perronciti[1] and first obtained in pure culture by Toussant,[2] Pasteur,[3] and Smith.[4] It is the cause of wide-spread epidemics among fowls and of material economic losses to poultry raisers. Especially investigated by Kitt.[5]

**Morphology.**—Small oval or biscuit-shaped bacilli measuring 0.25 by 0.3 to 1 $\mu$ in size. The organisms may be almost round at some times or somewhat more elongated at others. They are arranged as single cells, in pairs end to end, and rarely in short chains. In the fresh blood of infected animals chains of ten to twelve elements may occur (Perroncito). Wertheim[6] has found long chains in the tissues in chronic cases. Non-motile. No spores.

**Stains** by the usual anilin dyes, but not intensely. With methylene-blue, thionin, or crystal violet the organisms show polar staining, a deeply stained granule at each end, with a clear space between. The light central areas may be vacuoles (Nicolle). By intensive staining with hot carbolfuchsin after ether-alcohol fixation the entire organism can be stained. Gram-negative.

**Cultivation.**—The fowl-cholera bacillus can be cultivated easily from the blood or the organs of animals dead of the disease. The growth is very delicate and not unlike that of the streptococci. When associated with other organisms it may be isolated most easily by the inoculation of susceptible animals, pure cultures being obtained from the heart's blood. Growth under aërobic and anaërobic conditions and also under partial oxygen tension. Optimum temperature 37° C.

**Cultural Characters.**—*Agar Plates.*—Colonies grow slowly even at 37° C., appearing on the surface in two to three days, translucent, dull gray, $\frac{1}{2}$ to 1 mm. in size. Deep colonies are very fine and difficult to make out with the naked eye. Under low power the surface colonies

are pale, sometimes slightly yellowish, usually round and regular, with entire edges. Deep colonies round or disk-shaped. The surface colonies increase but slowly in size, and at the end of eight to ten days are seldom more than 1½ to 2 mm. in diameter.

*Plain Agar Slant.*—Delicate drop-like colonies, ½ to 2 mm. in diameter, appear in two to three days, seldom earlier. They do not expand much and are apt to remain isolated. Rarely the colonies coalesce in four to five days, forming a thin homogeneous expansion like the growth of a streptococcus, but somewhat more delicate.

*Plain Agar Stab.*—Slightly iridescent whitish streak in two to three days along the line of inoculation, gradually becoming somewhat opaque. Little or no surface expansion at the point of puncture.

*Gelatin Plates.*—Surface colonies develop slowly, appearing only in four to five days, delicate and translucent, about ½ to 2 mm. in size. Under low power round and regular with entire edges. Deep colonies are very small and difficult to see without magnification. The colonies remain small and delicate even in old plates. No liquefaction.

*Gelatin Stab.*—Delicate transparent, hyaline, whitish-gray colonies along the line of inoculation, often iridescent, appearing in four to five days. These coalesce to a whitish streak without processes. On the surface a delicate transparent expansion develops with wavy outlines, 1 to 2 mm. in diameter. The growth remains delicate even in old cultures. No liquefaction.

*Blood-serum.*—Very thin, delicate transparent expansion in four to five days. Later, in seven to eight days, it becomes dull white.

*Potato.*—Little or no growth on acid potato. On potato naturally alkaline, or slightly alkaline from washing in soda solution, certain strains may produce grayish-white colonies.

*Neutral or Alkaline Broth.*—Uniform turbidity developing in three to four days. This gradually clears and a viscid sediment forms. Occasionally a delicate film appears on the surface and along the walls of the tube. Sediment generally somewhat scanty. The same type of growth occurs in broth containing blood or serum.

*Litmus Milk.*—Rapid multiplication without change in reaction or coagulation.

*Litmus whey* of Petruschky. No acid.

Indol produced by some strains, not produced by others.

Slight acid from *dextrose* and *saccharose*, none from lactose.

Hadley[7] has reported a saccharose-negative strain.

**Resistance.**—Organisms survive several weeks and even three months in the organs of infected animals, fifteen to eighteen days in intestinal contents. Solid cultures may remain alive and virulent six months if kept from drying. Broth cultures show loss of virulence even if frequently transferred (Kitt). The organisms survive eight days in distilled water, thirty days in spring water. When dried on silk threads they lose virulence in seventy-two hours. In large pieces of organs like the liver living and virulent organisms may be found after three weeks. Killed almost instantly by disinfectants—1–5000 sulphuric acid, 1–500 hydrochloric acid, 1 to 2 per cent. carbolic acid, 1–500 potassium permanganate, 1–100 milk of lime.

**Isolation.**—Isolated easily from the blood of fowls dead of fowl-cholera, being present in the blood in enormous numbers, frequently inside the white cells. The organisms are present also in the internal organs, spleen, liver, and lungs, in the various exudates, in the urine, and in the intestinal contents. In chronic cases the bacilli are much more difficult to find in the blood and organs. When associated with other organisms isolation is best accomplished by the inoculation of a susceptible animal and cultivation from the heart's blood at autopsy.

**Pathogenic** to fowls, pigeons, ducks, geese, rabbits, house mice, field-mice, woods mice, guinea-pigs, and rats; less so to dogs and cats and large animals. Infection may be produced by direct contact of the organisms with the uninjured surfaces of the conjunctiva of the eyelid and the nasal mucosa, and also by direct contact with the uninjured skin. Infection is more certain when slight wounds are produced by scarification of the skin or by slight tearing of the cornea or the conjunctiva. Subcutaneous inoculation nearly always leads to infection, even with small quantities. V. Stang[8] says that one to six organisms, or 0.000001 c.c. of a broth culture, suffice with a susceptible animal. Pigeons are highly susceptible and are most useful for diagnostic purposes. A minute quantity of the organisms introduced on the point of a small knife beneath the skin over the breast muscle. Kills pigeons in twelve to twenty-four hours with characteristic lesions. At the point of inoculation there is a turbid, whitish-yellow, knotty thickening of the skin with a straw-yellow exudate below about the size of a silver coin. Beneath this the muscle is discolored, turbid, and fatty to a depth of ½ to 1 cm. In addition, the animals have a severe enteritis, the intestinal contents are very bloody, and an acute serous pericarditis is found. The spleen is greatly enlarged. The organisms are present in pure culture in the heart's blood and in blood from the various organs. With less virulent cultures the pigeons may live five to six or eight to ten days, showing at autopsy a general emaciation and anemia, usually a much enlarged spleen. With material contaminated with other organisms much the same type of infection occurs, the fowl-cholera organisms overgrowing the other bacteria and producing a characteristic result.

In fowls inoculated in the breast muscle death occurs in one to four days. At autopsy there is a wide-spread swelling at the point of inoculation, the connective tissue is swollen and

jelly-like, the muscle turbid, grayish white, or reddish-white. Occasionally a yellowish fibrinous exudate may be found beneath the skin. If the inoculation is on the wing there is a swelling at this point with an opaque and fatty discoloration of the skin and muscle, more or less limited and surrounded by a hemorrhagic zone. The fowls which die show pericarditis with hemorrhages, serofibrinous and fibrinous pneumonia and pleurisy, and a hemorrhagic gastro-enteritis. With more resistant animals or less virulent organisms an inflammatory exudate, dry and caseous, appears at the site of inoculation. Occasionally small abscesses or sloughs may form, with the separation of a dirty-brownish or brownish-green sequestrum, which may be 2 to 5 cm. long. The joints of the feet may be involved with a deforming arthritis which prevents the animal from walking (Lignières).

With ducks and geese the local changes are slight, but ecchymoses are found on the serous surfaces and an enteritis.

Rabbits are very sensitive. A small quantity of blood or of a culture rubbed on the slightly wounded surface of the inner side of the ear leads to an infection which is fatal in ten to twenty hours. According to Hertel 0.0000001 c.c. of a broth culture suffices to produce infection, especially when dropped into the conjunctival sac, from which it trickles into the nose. Rabbits may likewise be infected by rubbing the culture on the shaven skin. Spontaneous infections also occur in rabbits (Lignières, Smith). Young guinea-pigs die from large doses injected subcutaneously, 0.2 to 0.5 c.c. Old animals develop abscesses which break down, the pus containing viable organisms for many months. The abscesses may heal and the animals recover or the animals may die of septicemia. Intraperitoneal injection leads to a toxic infection and the virulence of the organism may be so increased that the animals may die in four hours (Lignières).

Rats are sensitive to intraperitoneal inoculation, not to subcutaneous.

Dogs and cats are not sensitive to subcutaneous inoculation, but die from intravenous inoculation in two to eight hours with a hemorrhagic enteritis. Swine, sheep, goats, cattle, and horses develop only local inflammation from subcutaneous inoculation, fatal septicemia from intravenous administration.

Infection can be produced by feeding the blood, organs, and intestinal contents of dead animals or cultures. Such infections are produced easily in pigeons, ducks, and geese, seldom in fowls, promptly in rabbits, but not in dogs, cats, or large animals, swine, sheep, goats, cattle, and horses.

In man local abscesses may develop when the organisms come in contact with wound of the skin (Marchiafava and Celli).

The filtrates of cultures are toxic, as first shown by Pasteur. Cultures filtered through Chamberland bougies contain a narcotic poison putting animals to sleep. This narcotic substance was subsequently obtained by Smith and by v. Stang.

Animals can be actively immunized against fowl-cholera (Pasteur) and vaccines have been widely used to prevent the disease. The serum from immunized animals also has some protective value. Hadley has reported an avirulent strain for adult rabbits, which possesses marked immunizing power against infection with virulent strains.

1. Perroncito: Arch. f. Tierheilk., 1879, 22.
———: Rev. de méd. vét., 1890, 523.
2. Toussant: Compt. rend. Acad. d. sc., 1879, lxxxviii, 1127.
3. Pasteur: Compt. rend. Acad. d. sc., 1880, xc, 239.
4. Salmon and Smith: Rep. Com. Agric., 1880, p. 401. *Ibid.*, 1882, p. 272.
5. Kitt: Geflügelcholera. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl., 1913, vi, 37.
6. Wertheim: Arch. f. exper. Path., 1889, xxvi, 61.
7. Hadley: J. Bact., 1919, iv, 65.
8. Stang: Zur Kenntnis der Toxin-bildung des *Bacterium avicidum.* Inaug. Dissert. Karlsruhe 1, B, 1901.

### PASTEURELLA LEPISEPTICA (Flügge)

First isolated in pure culture and described accurately by Koch[1] in the disease rabbit septicemia first noted by Davaine.[2] It is also known as *Bacillus bipolaris septicus*, *Bacillus lepisepticus*, and *Bacillus cuniculicida.*

**Morphology and Staining Reactions.**—In the blood and tissues of infected animals minute oval bacilli, measuring 0.3 to 0.5 $\mu$ in breadth by 0.8 to 1 $\mu$ in length. Longer forms measuring 1.5 $\mu$ are rarely seen. Stained with the usual aqueous anilin dyes, gentian-violet, methylene-blue, and carbolfuchsin. Polar granules, deeply stained, lie at each end of the organism with an unstained area between. These are best demonstrated by slightly warming the preparations during staining and then rapidly decolorizing in $\frac{1}{2}$ per cent. acetic acid or alcohol. Good polar staining by Giemsa. Gram-negative. Gram-Weigert negative. In cultures the organisms may also be stained by aqueous anilin dyes. They appear as round or oval cocci, diplococci, and short bacilli uniformly stained, together with short threads which rarely show

polar granules. If films are stained with carbolfuchsin one-half to one second, without previous fixation by heat, great diversity in morphology and staining reaction is apparent. Uniformly stained organisms appear, intermingled with short rods which have sickle-shaped unstained areas along the sides. The majority are deeply stained at one end, almost colorless at the other, showing only a very fine outer rim of cell wall. Others show the characteristic double polar staining. Rarely short chains are found consisting of elements showing both unipolar and bipolar staining. In old cultures the organisms stain irregularly and the polar granules are less definite.

**Cultivated** easily on ordinary media. Growth very fine and delicate like that of the streptococci. Optimum temperature 37° to 38° C. Growth also at 12° to 13° C. and at 42° to 43° C. Development under aërobic conditions better than under anaërobic conditions. Growth also under partial oxygen tension. Little growth in neutral or acid media, best development on alkaline media.

*Plain Agar Plates.*—Colonies appear on the surface usually in forty-eight hours, ½ to 1 mm. in size, round, gray, and transparent. Under low power the centers are dark, peripheries light, edges entire. Deep colonies very minute, barely visible, under low power usually round or oval. In three to four days the colonies become somewhat opaque, larger when separated, measuring 1 to 2 mm. in diameter. They never grow much beyond this size.

*Plain Agar Slant.*—Isolated colonies in two to three days coalescing to a gray transparent moist layer. The growth increases somewhat in intensity and tends to run down the surface. The water of condensation becomes turbid with a grayish finely flocculent viscid sediment.

*Plain Agar Stab.*—Delicate growth along the line of inoculation, slight or no surface expansion, in two to three days. Little increase in intensity even in old cultures.

*Gelatin Plates.*—Colonies appear in two to three days, ½ to 1 mm. in size, round, gray, and transparent. Under low power the centers are dark, peripheries light, edges wavy. In eight to ten days the colonies increase slightly in size and become somewhat opaque.

*Gelatin Stab.*—Fine granular deposits appear along the line of inoculation in two to three days, later coalescing to a finely granular whitish streak without peripheral processes. On the surface a thin delicate expansion with jagged edges.

*Blood-serum.*—Delicate dry opalescent expansion.

*Potato.*—No growth on acid potato. Grayish-yellow colonies on alkaline potato.

*Broth.*—Uniform turbidity in two to three days. The broth clears and a flocculent viscid sediment forms. On shaking, this rises in delicate streaks. Occasionally a delicate iridescent film appears on the surface. Many strains grow only in the form of a flocculent sediment, the fluid remaining clear. More abundant growth in broth with serum added.

*Litmus Milk.*—No change in reaction.

*Special Media.*—Delicate growth on lactose litmus agar without change in reaction. Rather abundant, raised, gray, moist growth on blood agar without hemolysis. No growth on Endo-agar or malachite green agar.

**Pathogenic Action.**—Organisms isolated from rabbits differ greatly in virulence both for rabbits and for other animals. Rabbits, however, are so highly susceptible to organisms of this group from other animals that almost any type may be found in them. The rabbit organisms have apparently no specificity in their action, but rather the general pathological characteristics mentioned above.

1. Koch: Wundinfektionskrankheiten. 1878.
2. Davaine: Bull. Acad. de méd., Sept. 17, Oct. 8, 1872, April 29, 1873.

### PASTEURELLA BOVISEPTICA (Kitt)

First isolated by Kitt[1] from a disease of cattle described by Bollinger[2] as "Wild-und Rinderseuche." Recognized in America by Smith.[3]

Short, non-motile encapsulated rods which fail to grow on potato, and do not change the reaction of milk. Acid in dextrose and saccharose, but not in lactose. Phenol in old broth cultures. Indol variable. Pathogenic for rabbits in 0.1 to 0.2 c.c. doses.

1. Kitt: Sitzungb. d. Gesellsch. f. Morphol. u. Physiol. München, 1885, p. 140.
2. Bollinger: Ueber eine neue Wild-und Rinderseuche welche im Sommer 1878 in der Umgebung von München beobachtet wurde. Munich, 1878.
3. Smith: Rep. Bureau Animal Indust., 12 and 13, 1895–96.

### PASTEURELLA SUISEPTICA (Loeffler and Schütz)

Observed by Loeffler[1] on the skin and in the organs of swine dead of swine-erysipelas and later accurately described by Loeffler and Schütz.[2] Known also as *Bacillus suisepticus*.

Fig. 111.—*Bacillus bovisepticus* from plain agar twenty-four hours old, stained with gentian-violet.

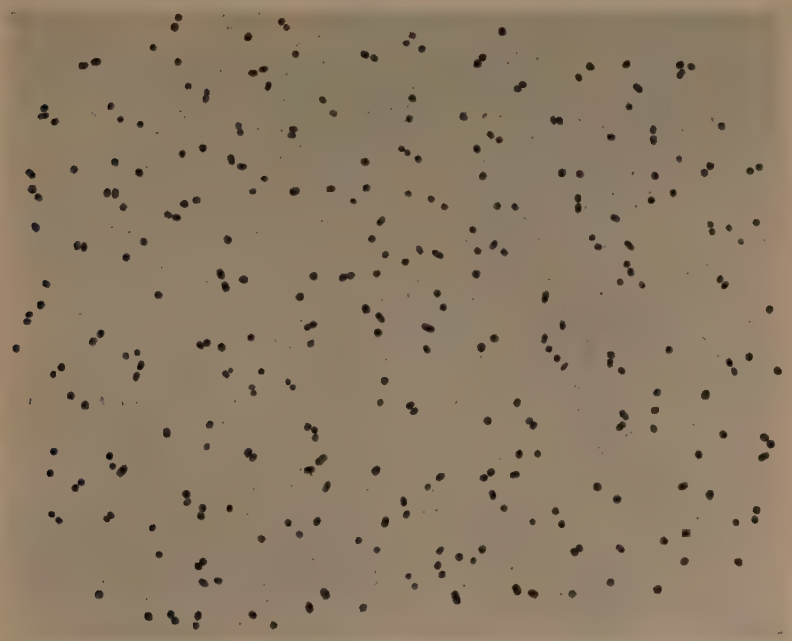

Fig. 112.—*Bacillus bovisepticus* from dextrose agar twenty-four hours old, stained with gentian-violet.

**Morphology.**—Short oval bacilli in infected tissue, measuring 0.4 to 0.6 $\mu$ in width by 1.2 to 1.4 $\mu$ in length (Uhlenhuth and Haendel[3]). In the cultures the organisms are often smaller, measuring 0.3 to 0.4 $\mu$ in breadth by 0.7 to 1 $\mu$ in length. Coccus-like forms and somewhat longer rods are common. Arranged as single individuals, pairs end-to-end, and short chains. Non-motile. No spores.

Fig. 113.—*Bacillus bovisepticus* from spleen of infected rabbit, stained with carbolfuchsin.

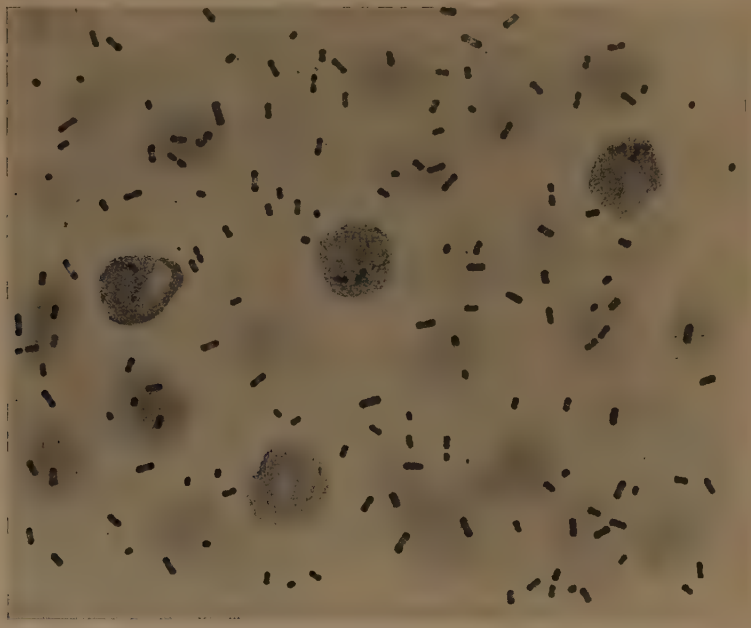

Fig. 114.—*Bacillus bovisepticus* from heart blood of infected rabbit, stained with methylene-blue.

**Stains** well with anilin dyes, gentian-violet, methylene-blue, and dilute carbolfuchsin. In the animal body the bacilli show polar granules, best brought out by fixing in alcohol and staining with borax methylene-blue, by long exposure (five minutes) to dilute aqueous or aqueous alcoholic methylene-blue ($\frac{1}{2}$ to 1 per cent.) followed by washing one to two seconds in

0.5 per cent. acetic acid. Polar staining also brought out by Giemsa. In cultures polar granules are not so pronounced and may not be demonstrable.

Smith[4] reports that the organisms in the animal body possess a definite capsule. Preisz[5] has reported encapsulated organisms in agar cultures demonstrated by Loeffler's flagella stain.

*Cultivated* easily on ordinary media. Growth delicate, somewhat like that of the streptococci. Optimum temperature 30° C. Growth range from 8° to 42° C. (Frosch[6]).

**Cultural Characters.**—*Plain Agar Plates.*—Surface colonies small, round, shimmering, transparent, about ½ to 1 mm. in size in twenty-four hours. Under low power circumscribed, with smooth margins, sometimes nucleated. Deep colonies barely visible in twenty-four hours; under low power spherical or biconvex disks. The colonies increase in size, but slowly, and at the end of four to five days may be 1 mm. in diameter. They retain about this size even in old plates.

*Plain Agar Slant.*—Thin, delicate, bluish-white or transparent expansion usually after forty-eight hours. In four to six days the growth spreads somewhat, especially in the lower part of the tube near the water of condensation. In some cultures the growth may be viscid and slimy. According to Preisz this is asssociated with the presence of a capsule about the organism.

*Agar Stab.*—Delicate growth along the line of inoculation in two to three days with little or no surface expansion. After seven to eight days the line growth is more opaque, but there is little or no surface development.

*Dextrose Litmus Agar Plates.*—Small, delicate, surface colonies, in two to three days measuring ½ to 1 mm. Under low power round or elliptical, slightly granular, translucent, edges entire. Deep colonies barely visible. Under low power oval or disk-shaped. The colonies remain small and delicate and the agar develops a faint acid reaction.

*Dextrose Litmus Agar Slant.*—Delicate expansion appearing in two to three days. Agar slightly acid. The growth remains delicate even in old cultures.

*Lactose Litmus Agar* (v. Drigalski-Conradi).—Small, delicate, transparent, bluish colonies. *Neutral red agar* not decolorized.

*Gelatin Plates.*—Thin, delicate, bluish-white, transparent colonies, visible usually only after three days, almost pin-point in size. Under low power round, regular, uniform, often showing concentric rings of growth. Older colonies increase slightly in size, but are seldom more than ½ mm. in diameter. No liquefaction.

*Gelatin Slant.*—Very thin, delicate, whitish, transparent growth in three to four days.

*Gelatin Stab.*—Slight growth along line of inoculation and very delicate surface growth in three to four days. Little increase, even in old cultures. No liquefaction.

*Blood-serum.*—Grayish-white, dry growth in two to three days, sometimes more abundant than on agar. Development remains scanty.

*Potato.*—On acid potato no growth. On alkaline potato a faint whitish or brownish growth may occur or growth may fail.

*Broth.*—Faint turbidity and delicate sediment in three to four days. The sediment may be grayish-white, viscid and slimy, rising in coherent threads on shaking. Growth remains moderate.

*Litmus Milk.*—Development without change in reaction.

*Litmus whey* of Petruschky. No change.

*Dextrose fermentation tubes* show faint turbidity and sediment in the bowl in two to three days, with a turbidity in the closed arm. Reaction faintly acid (Smith, Dorset[7]). Faint acidity also from saccharose but not from lactose.

Indol and phenol produced in peptone solutions (Smith, Joest,[8] de Schweinitz[9]). Nitrates reduced to nitrites (Voges and Proskauer[10]). Hydrogen sulphid produced in peptone media containing ammonium sulphate.

*Resistance.*—Swine-plague dies out rapidly on artificial media, especially if the media becomes dry. Easily destroyed by drying on silk threads, in forty-two hours in the light, seventy-two hours in the dark. Killed by direct sunlight in six to eight minutes, by diffuse daylight in an hour (Beck and Koske[11]). Survive only seven to ten days in water and in the upper layers of the soil. Survive fourteen to forty-five days in dung at a depth of 20 cm. (Beck and Koske). Destroyed easily by heat, 58° to 60° C. in fifteen to twenty minutes (Salmon and Smith[12]), at 70° C. in fifteen seconds (Junack[13]). Survive up to five days at low temperatures. Destroyed by the usual disinfectants in a short time.

**Pathogenic Action.**—The virulence of different cultures varies greatly and of the same culture, being lost by cultivation and restored by animal passage. Virulence lost rapidly, according to Joest, in one-half to one hour by exposure to diffuse daylight; in five minutes by exposure to sunlight. Virulence also lost at low temperature and above 37° C. Mice and rabbits are especially sensitive to inoculation, fetal infections developing from cutaneous, subcutaneous, intravenous, and intraperitoneal inoculation. With virulent cultures 1/1000 to 1/1,000,000 c.c. will produce fatal septicemia in sixteen to eighteen hours. With intravenous and intraperitoneal inoculations the death may occur even earlier. With cutaneous and subcutaneous inoculation there is a marked local reaction, especially in rabbits, with

wide-spread edema and infiltration. At autopsy there is a bloody edema at site of inoculation, swelling of the adjacent lymph-glands and spleen, parenchymatous degeneration of liver and kidneys, exudates in the abdominal cavity, hemorrhagic edema of the lungs, hemorrhages in the serous membranes and heart muscle. The organisms are everywhere; at site of inoculation, in the blood, in the organs, and in the exudates. Inhalation, intratracheal and intrapulmonary inoculation lead to infection in rabbits with early death. The animals may show croupous pneumonia with fibrinous pleurisy which leads to general infection. Infection may be produced by intracerebral inoculation (Beck and Koske), by inoculation into the anterior chamber of the eye, rarely by inoculation of the conjunctiva of the eyelid, and occasionally by feeding (Karlinski[14] and Lignières[15]). Mice are susceptible in the same way as rabbits, also per os (Preisz, Karlinski, etc.).

Guinea-pigs are less sensitive than mice and rabbits. Highly virulent cultures produce fatal infections by intraperitoneal and subcutaneous inoculation, especially in young animals. With less virulent cultures subcutaneous inoculation produces local abscesses (Schütz, Beck, and Koske). Cutaneous injection does not lead to infection; inhalation and feeding do.

Rats are usually immune. They can be killed by intraperitoneal inoculation, not by subcutaneous (Lignières).

In pigeons and hens infection by subcutaneous or intramuscular inoculation occurs only from large doses of virulent cultures. Intramuscular infection more certain than subcutaneous (Smith). An extensive necrosis of the muscle develops at the point of inoculation, also an enlargement of the spleen with inflammatory changes in the mucus membrane of the stomach and intestine. Organisms are abundant at the site of inoculation, sparse in the blood and organs. Both pigeons and hens can also be infected by feeding and show lesions like those from fowl-cholera. Sparrows susceptible to feeding (Koske).

Large animals (beeves, goats, sheep, dogs, cats, horses, and asses) are sensitive to swine-plague. Cattle may die in three days, goats and sheep in eighteen to twenty-four hours from intravenous inoculation, calves in six hours (Lignières). From subcutaneous injection local changes only occur in beef, sheep, wethers, and goats. Young dogs die from large doses in nine to thirty hours from intraperitoneal injection (Prettner[16]), cats in five hours (Lignières). Dogs and cats are almost immune to subcutaneous inoculations showing only local changes. Dogs not infected by feeding.

With horses and asses subcutaneous inoculation is followed by wide-spread edema and infiltrations and occasionally abscesses, many organisms being present in the pus. Intravenous inoculation produces a severe, often fatal, poisoning (Schreiber,[17] Joest, Beck, and Koske). The animals may develop chronic intoxication from repeated injections, with jaundice, stomach and intestinal catarrh, and attacks like cholera morbus.

In swine cutaneous inoculation does not lead to infection. Subcutaneous inoculation with virulent cultures leads to rapidly fatal hemorrhagic septicemia with death in twenty-two to sixty hours. At autopsy there is a hemorrhagic edema at site of inoculation and a septicemia with organisms everywhere in the blood and organs. When cultures are less virulent only local edema and infiltration with abscess formation may be seen, the animals recovering, or a chronic infection may develop, lasting three to four weeks. At autopsy various lesions may be found, fibrinous pleurisy, patches of pneumonia, necrotic foci in the lungs, enlargement of the spleen, fatty degeneration or inflammatory changes in the liver. Atelectatic areas may be present in the lungs in chronic cases, containing the specific organism. Intraperitoneal inoculation may produce fatal infection in nine to fourteen hours (Salmon and Smith). With intravenous inoculation the swine die in twenty-four hours of septicemia. With small doses or less virulent cultures lesions develop in the various organs of the body, in the lungs and occasionally in the joints.

Infection by feeding does not occur. By intrapulmonary injection a multiple necrotizing pneumonia can be produced (Salmon and Smith, Schütz), as well as by inhalation (Schütz) and by intratracheal inoculation (Welch,[18] Smith).

Soluble poisons are not produced by *Bacillus suisepticus* (de Schweinitz, Smith and Moore). Old killed cultures are somewhat poisonous to mice (Voges and Selberg), probably from an endotoxin. Beck and Koske find that cattle are very susceptible to this endotoxin which is easily destroyed.

Organisms not differing in morphology and cultural reactions are present on the mucous membrane of the nose and throat and in the saliva in a large number of healthy swine (66 positive out of 120 examinations (Uhlenhuth). Considerable discussion has arisen as to whether the epidemics of swine-plague are to be traced to infection by these sputum bacteria. The natural infection seems to occur both through the respiratory tract and through wounds of the skin.

Active immunity against swine-plague organisms has been produced by Smith and Moore,[19] who used as vaccines living cultures or cultures killed at 58° C. by de Schweinitz, who employed for immunizing an albuminose from the organism by Greither[20] who used filtrates from old broth cultures, and by Klett and Braun,[21] who employed mixtures of filtrates and the poisons of old cultures. The serum of properly immunized animals has protective properties against infection in other animals. Such sera have been used largely in veterinary practice both alone and in combination with vaccination with bacillus extracts.

1. Loeffler: Arb. a. d. K. Gsndhtsamte., 1886, i, 46.
2. Schütz: Arb. a. d. K. Gsndhtsamte., 1886, i, 56, 376.
3. Uhlenhuth and Haendel: Schweinepest and Schweineseuche. *In:* Hanb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl., 1913, vi, 325.
4. Smith: Special report on the cause and prevention of swine plague. Washington. 1891.
5. Preisz: Ztschr. f. Tiermed., Bd., ii.
6. Frosch: Ztschr. f. Hyg., 1890, ix, 235.
7. Dorset: Bureau of Animal Industry, Circular 43, 1904.
8. Joest: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1903, iii, 576. *Ibid.*, 1904, iv, 1216.
9. de Schweinitz: Rep. Bureau Animal Indust., 8, 1891. *Ibid.*, 9, 1892.
10. Voges and Proskauer: Ztschr. f. Hyg., 1898, xxviii, 20.
11. Beck and Koske: Arb. a. d. K. Gsndhtsamte., 1905, xxii, 429.
12. Salmon and Smith: Rept. Bureau of Animal Indust., 1885.
13. Junack: Monatschr. f. Prakt. Tierh., 1904.
14. Karlinski: Ztschr. f. Hyg., 1898, xxviii, 373.
15. Lignières: Contribution à l'étude des septicæmies hémorrhagiques. Buenos Aires, 1900.
16. Prettner: Ztschr. f. Fleisch-u. Milchhyg., 1900.
17. Schreiber: Berl. tierärztl. Wchnschr., 1899, etc.
18. Welch: Johns Hopkins Hosp. Bull., 1889, i, 9.
19. Smith and Moore: Additional investigations concerning infectious swine diseases. Washington, 1904.
20. Greither: Ueber Immunisierung gegen Swine plague und Hog cholera mittels Immunproteiden. Inaug. Diss., 1901.
21. Klett and Braun: Deutsche tierärztl. Wchnschr., 1904.

### PASTEURELLA VITULISEPTICA (Schirop)

Originally isolated by Schirop[1] from pneumonia in calves. Studied especially in America by Jones[2] who investigated 16 strains from pneumonia in cattle and found that they fell into 3 groups.

Group 1 (8 strains). Short, non-motile, Gram-negative encapsulated rods which fail to form indol and produce clear zones about deep colonies in horse blood agar plates. Acid in dextrose, lactose, saccharose, maltose, and mannitol. Final hydrogen-ion concentration in dextrose broth lies between pH 6.3 and 6.1.

Group II (6 strains). Ferment dextrose and saccharose, do not attack lactose, maltose or mannitol. All produce indol. All soluble in ox or guinea-pig bile. None hemolytic.

Group III. Like Group II, but attack mannitol as well as dextrose and saccharose. Produce indol, but are not bile soluble.

Immunological groupings follow cultural reactions.

1. Schirop: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1908, xlviii, Orig. 1, 307.
2. Jones: J. Exper. M., 1921, xxxiv, 561.

### BACILLUS PESTIS Kitasato and Yersin

Discovered almost simultaneously by Kitasato[1] and by Yersin[2] in Hongkong and usually put in the group of hemorrhagic septicemia because of its morphology and pathogenic properties. Always found in the disease and never isolated from normal individuals.

**Morphology and Staining Reactions.**—In smears from infected tissues in man and animals short, plump rods, round at both ends, slightly thicker in the middle. When stained the poles of the organism take the dye more deeply than the central portions. Usually single elements or in short chains. Long chains and threads rare. Breadth 0.5 to 0.7 $\mu$, length 1.5 to 1.75 $\mu$ (Ghon and Sachs). The length varies greatly, and short oval rods and long rods are frequent. In addition many changes in morphology are encountered, pale, irregular, bulging forms, bladder-like disk-shaped organisms and yeast-like elements taking the stain only at the periphery. One can often make out a series of gradations from the usual bacillary form to the bladder-like organisms. According to Albrecht and Ghon,[3] Dieudonné, and Otto these irregular forms are still possessed of infectivity. They are especially common in cadavers some time after death. This tendency to produce involution forms must be regarded as a diagnostic characteristic of *Bacillus pestis*. In films made from cultures the organism appears as short rods with frequent threads, which are plumper than the rods and show no distinct segmentation. The morphology is usually more regular, but the organism shows the same tendency to involution, producing irregular, bulging, swollen, and bladder-like forms, especially on un-

Fig. 115.—Film from culture of *Bacillus pestis*, stained with carbol-thionin.

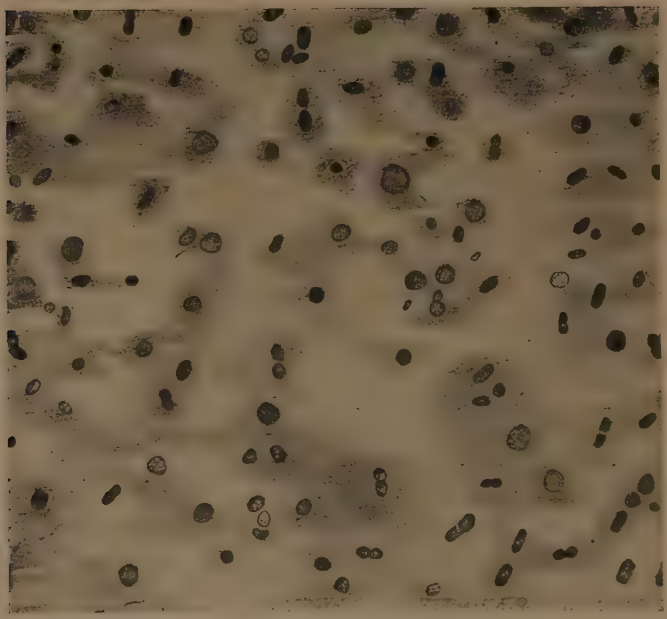

Fig. 116.—Film from salt agar culture of *Bacillus pestis*, forty-eight hours old.

Fig. 117.—Film from salt agar culture of *Bacillus pestis*, showing swollen involution forms.

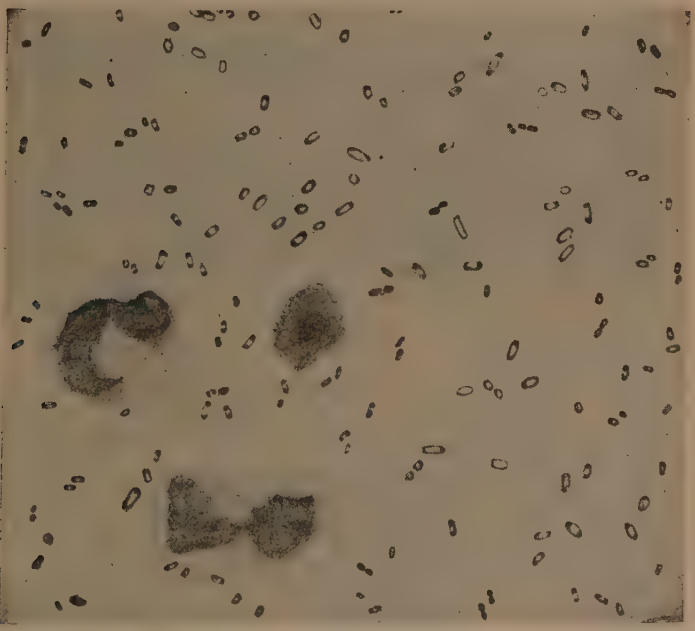

Fig. 118.—*Bacillus pestis* in peritoneal exudate of guinea-pig, stained with carbol-thionin.

favorable media. Club-shaped organisms and true branching have been observed by Albrecht and Ghon and by Kolle.[4] *Bacillus pestis* can be stained by the ordinary anilin dyes, best with methylene-blue or strong carbolfuchsin immediately washed off with water (Gottschlich[5]). Good preparations showing polar staining are often procured by first treating the films one-half minute with ½ per cent. acetic acid. In addition to the organisms in which the poles are stained, the stained areas may be on one side and the unstained areas on the other (Gaffky[6]).

Good results are also obtained by treating the film with absolute alcohol, letting it evaporate by holding in the flame, and then staining with dilute aqueous or alkaline methylene-blue, dilute carbolfuchsin, or borax methylene-blue. For organisms in tissues the sections should be fixed in alcohol or sublimate and then stained in alkaline methylene-blue or Unna's polychrome methylene-blue. By Romanowski's method the organisms appear as dark blue-violet rods on a rose-colored background and frequently show good polar granules. Always decolorized by Gram's method both from the tissues and from cultures. Non-motile. No flagella. No spores.

*Bacillus pestis* has a viscid capsule. This is best shown in smears from the peritoneal exudate of guinea-pigs and mice. In smears from cultures capsules are easily demonstrated

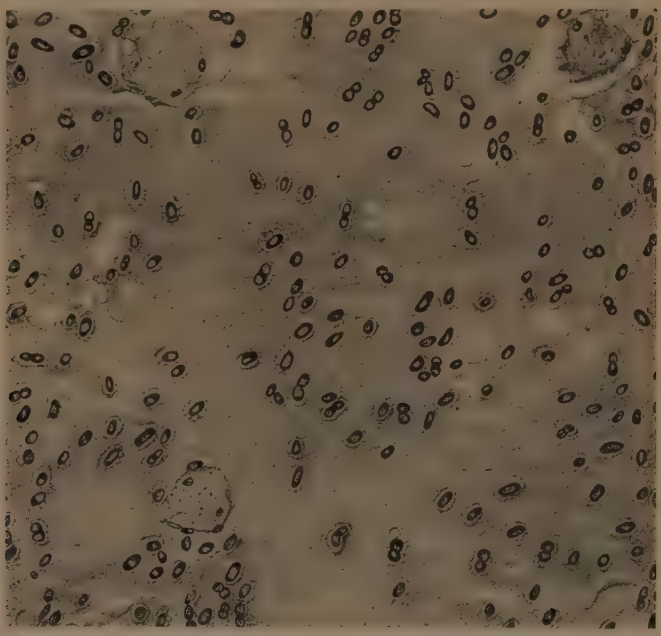

Fig. 119.—*Bacillus pestis* from rat liver, stained with carbolfuchsin.

by Loeffler's flagella stain (Zettnow[7]). Better results may be obtained by alcohol fixation than by fixation by heat.

In cultures four years old and still capable of multiplication Schultz has observed peculiar granules which seem to arise from thickening of the protoplasm. On transfer to agar abundant growth of *Bacillus pestis* occurs. These granules are not spores, since they are destroyed by heating to 50° C. in one and three-quarter hours.

On *salt agar*, 2½ to 3½ per cent. the morphology is especially characteristic (Hankin and Leumann[8]). In twenty-four to forty-eight hours the cultures show swollen spherical spindle-shaped and oval forms which often resemble yeast cells. These structures may take the stain deeply or barely at all. They also occur on very dry agar, but not so frequently (Haffkine[9]). They show best on dry agar with 3 to 4 per cent. NaCl.

Similar involutions forms have been described in a great many organisms, *Bacillus pyocyaneus*, the choleræ vibrio, *Bacillus acidi lactici*, etc. Higher concentrations of salt and more long-continued growth is necessary before such forms appear. According to Kessel and Overbeck the only organisms which show these swollen forms are *Bacillus lactis aërogenes* and Hofer's *Bacillus of crab plague*. With neither culture are the involution forms the same as with *Bacillus pestis*. Pfeiffer[10] has tested a large series of organisms and found that the species which produced the most exaggerated involution forms did not produce any which

Bacillus pestis, smear from rat liver, stained with carbolfuchsin.

could not be differentiated from those of *Bacillus pestis*. Recently Smillie has described an organism of the hemorrhagic septicemia group which shows large swollen forms on salt agar.

In broth cultures the plague bacilli appear in long chains of coccoid elements resembling streptococci, close observation being required to show that these coccoid elements are in reality short bacilli. They are often in straight lines or bent at rather sharp angles. According to Loeffler involution forms show up well in broth with carbolic acid added, and in strong concentrated broth.

Kodama has described true branching in the plague bacillus on coagulated egg-albumin.

**Cultivation.**—*Bacillus pestis* grows best at 25° to 30° C. and poorly above 35° C. It grows well at room temperature and slowly at 12° to 15° C. Growth occurs, but is slow at the temperature of the ice-box, 4° to 5° C. The upper limit of growth is 43.5° C. All cultures grow slowly, especially those from infected tissues. The media should be moist, neutral, or slightly alkaline. No growth in strongly alkaline media or in acid media. Neither glycerin nor glucose favor the growth.

The organism is strongly aërobic, requiring free atmospheric oxygen for its development in cultures. Anaërobic growth is usually very scanty when it does occur (German Commission).

**Cultural Characters.**—*Neutral* or *Slightly Alkaline Agar*.—Colonies in eighteen to twenty-four hours at 30° C. small, delicate, dewdrop-like, grayish-white in reflected light, bluish in transmitted light. Under low power the colonies are coarsely granular, show dark centers surrounded by delicate homogeneous indented peripheries. The edges are circumscribed and sharply limited. In forty-eight hours the colonies are larger, gray by reflected light, whitish-gray by transmitted light. They appear as more or less transparent irregular expansions with granular contents, the centers prominent and dark, the edges broad, delicate, jagged, or indented. In addition to these typical colonies atypical colonies appear, some with smooth edges, others with large granules, while in many instances the colonies are quite large (several millimeters Yersin). Deep colonies not characteristic.

*Agar Slant*.—Delicate grayish-white expansion at 30° C. in thirty-six to forty-eight hours, viscid and slimy. When touched with the platinum needle long threads are brought away. The culture does not adhere to the medium.

*Agar Stab*.—Abundant growth along line of inoculation with tuft-like protrusions in old cultures.

*Salt Agar*.—Two and one-half to 3½ per cent. Growth like that on ordinary agar, but less abundant. On 3 to 4 per cent. salt agar the growth is very scanty (Zlatogoroff[11]).

*Gelatin plates* are especially valuable for the cultivation of the plague bacillus from mixtures, as in sputum, feces, urine, etc. The gelatin should be slightly alkaline like alkaline agar. In two to three days at 22° C. the colonies are visible as fine, half-transparent points, at first gray, then yellow. The center of the superficial colonies is raised over the surface of the medium, is coarsely granular, and surrounded by a small, delicate, light, somewhat jagged edge. Deep colonies non-characteristic. The characteristic shape of the gelatin colony is brought out by impression coverslips (Klatschpröparate). The edge is seen to consist of long threads wound together in a kind of coil. Such colonies are common when infected material is streaked over the plates.

*Gelatin Stab*.—Slow growing, delicate white streak along entire line of inoculation with occasional peripheral radiations, especially on soft gelatin and with old cultures (Albrecht and Ghon). No liquefaction.

*Gelatin Slant*.—Superficial expansion often with long tuft-like radiating processes.

*Broth*.—Growth in twenty-four hours at 30° C. as a delicate, flocculent whitish sediment from which fine floccules or streaks may extend up the wall of the tube. At the same time, when the broth is not moved, a whitish ring of growth develops on top along the wall of the container and gradually spreads over the surface as a thin floating film. The development of this film is favored by the addition to the broth of floating substances like drops of oil or butter to which the plague bacilli may attach themselves (Haffkine). They then develop in the form of stalactites hanging down from these foreign bodies and are met by similar processes of growth projecting from the mass below like stalagmites. On the slightest movement these stalactites settle to the bottom, the floccules break up and render the broth, previously clear, turbid. In old cultures this turbidity gradually clears.

*Bacillus pestis* grows abundantly in calf-meat infusion (Zlatogoroff), badly in fish infusion.

According to the Austrian Plague Commission the plague bacillus produces no acids, but alkali in ordinary broth. When this reaches a maximum corresponding to 1.5 to 2.5 sodium hydrate the development ceases.

No gas produced from sugars. The Indian Commission found that the plague bacillus produces acid in glucose, galactose, levulose, and mannite, none in lactose or dulcite.

Good growth in coagulated blood-serum and on Loeffler's serum, especially in water of condensation, where good chains are formed. Kossel and Frosch[12] and Overbeck find that the virulence of cultures is maintained better on Loeffler's serum than on agar.

Slow growth in milk without clotting. Slight development in Petruschky milk-whey with a faint reddening due to acid. Little growth in peptone solution. No indol. No hydrogen sulphid.

On potato a slow growth at 35° C., in forty-eight hours a thin, moist, whitish slightly raised expansion, at first dark then brownish-yellow. More marked on alkaline than on acid potato. The growth is more abundant at 15° to 20° C. than at 37° C. (Lignières[13]). No growth on cooked bananas. Grayish moderate expansion on cooked rice. In filtered sea-water growth as large cocci (Würtz and Bourges[14]).

Bielalowsky[15] has found that plague cultures are hemolytic, the hemolysin being resistant to heat and destroyed by boiling.

**Survival.**—Plague bacilli are able to survive in the stools of animals, but there is no reliable evidence of this in man. Ohwada[16] has observed virulent plague bacilli in the dejecta of resistant Japanese field-mice without the appearance of illness in the animals. Experimentally introduced they survive in the bodies of fishes and insects (ticks). Introduced per rectum they survive a long time in the intestine of immunized guinea-pigs (Fornario[17]). They have also been found in the dejecta of a dog fed upon them (Albrecht and Ghon).

In man they survive a long time. Thus v. Vagedes[18] found them after seven weeks in the sputum of a patient and in a pelvic abscess two and a half months old. In purulent buboes they are present in small numbers. Stricker cultivated them from this source four months after the onset. Strong[19] has found that virulent plague bacilli injected subcutaneously in apes were present after six to eight hours, but not recovered after twenty-four hours. In the bodies of rats plague bacilli survive especially well. In pus from buboes removed from the body and kept from drying they have been found for twenty days when other organisms were absent (Albrecht and Ghon). In sputum mixed with other bacteria they were found after ten days by the German Commission, but not after sixteen days. Artificially introduced into sterilized feces they survive four days, but not five days. They were found alive and virulent in the decomposing spleen of a plague cadaver after four days, but not after five days. In the decomposed body of guinea-pigs dead from intraperitoneal infection they were found three days after death by Gottschlich. In other instances they have been found after thirty days (Yokote[20]).

In cultures plague bacilli survive months and even a year when protected from light and drying. Schultz[21] found them alive after four years in broth cultures. At low temperatures, 20° C., they survive long periods. At 37° C. they lose viability rapidly. On foods when no other bacteria are present they survive two to three weeks (Gladin[22]), in milk three months, in rice eighteen days (Rosenau[23]). In polluted tap water they were not found by the German Commission after five days, in sterilized tap water after ten days. In sterilized earth they were isolated for two weeks when the organic material was destroyed, for twelve weeks when this was not destroyed, and for eight weeks in earth not sterilized (Gladin). In general plague bacilli have considerable resistance to putrefaction, but die out rapidly at higher temperatures and with an abundance of saprophytes.

**Destruction.**—Destroyed in four days at 28° to 30° C. when dried on coverslips (Kitasato), in three hours in the desiccator (Wilm). Survive six days on wool silk and gauze cloth, on silk threads five days, on filter paper and pieces of glass two days (German Commission). In dried pieces of organs no survival after seven days. Rapid drying very injurious to the plague bacillus. Sputum dried on wool contains virulent organisms after a month (Gottschlich), urine artificially infected and allowed to dry contained no living organisms after three days. Plague bacilli dried on fine particles of dust died out in short time (Germans[24]). The organisms survive drying much longer at 37° C. than at 22° C. and in thick layers longer than in thin. In the experience of Verjbitzki[25] plague bacilli in clothing soiled with the feces of fleas and ticks are alive and virulent after five months. Destroyed by sunlight in pus from buboes dried on coverslips in three to five hours (Kitasato and Wilm). Pure cultures destroyed in one to four hours when dried on coverslips (German Commission). Dried on coverslips and exposed to the sun of European climate destroyed in one hour (Abel[26]), two to three and one-half hours (Giaxa and Gosio[27]). The higher the temperature and the thinner the layer exposed to the air, the more rapid the destruction. On various objects exposed to sunlight they do not survive more than five to six hours. Cold has little effect upon plague bacilli. They grow at 4° C. (Forster), but not at 0° C., although not killed. They survive for days at temperatures below zero, but gradually lose virulence (Wladimioroff and Kresling[28]). They withstand alternate freezing and thawing forty days (Gladin).

In the frozen cadavers in Manchuria plague bacilli were alive after a year (Schouroupoff). Destroyed by dry heat at 100° C. when dried on coverslips (Abel). Not destroyed by heating to 50° C. one hour. In broth cultures killed at 80° C. in thirty minutes (Kitasato), at 100° C. in a few minutes. According to Abel killed at 100° C. in one minute, 80° C. in five minutes, 70° C. in ten minutes, 50° C. in sixty minutes. Not destroyed when heated over water-bath to 55° to 60° C. in one hour (Albrecht and Ghon), the material producing plague in guinea-pigs, even though not growing on media. Concentrated emulsions of plague bacilli killed only by an hour's exposure to 65° C. when shaken (Kolle). Killed easily by disinfectants, by 1 per cent. carbolic in two hours, 5 per cent. in ten minutes (Abel), by 1 per cent carbolic and sulphuric acid mixtures in ten minutes (Abel), by 0 to 2 per cent. solution in thirty minutes. Destroyed by 1 per cent. lysol in five minutes when dried on coverslips, by 1 per cent. sublimate in two minutes, by 1 per cent. milk of lime in two hours (Kitasato),

by chlorid of lime in five minutes (Abel), by sulphuric acid 1–2000 in five minutes (German Commission), by hydrochloric acid 1–1000 in thirty minutes, by formalin 1–50 in thirty minutes when dried on filter paper (Schultz).

**Pathogenic Action.**—The plague bacillus is virulent for a large series of animals, the action upon rats and guinea-pigs being quite characteristic and almost diagnostic for the organisms.

*Rats.*—Subcutaneous injection with minute quantities of virulent cultures produces a fatal infection. At autopsy there is a hemorrhagic exudate at the site of inoculation containing many plague bacilli, the neighboring glands are enlarged, and the remote glands often somewhat swollen. The spleen is greatly enlarged, reddish-black, and contains plague bacilli in enormous numbers. Lung and liver are hyperemic, peritoneum moist and glistening, the exudate scanty, but free of organisms. Infection is also produced by rubbing material containing the plague organisms on the shaved skin even when this is not bloody (Albrecht and Ghon). Intraperitoneal inoculation is fatal in a short time. The exudate is scanty, but contains encapsulated organisms. The spleen is enlarged and full of plague bacilli. The dose should not be too large, otherwise the animals die of toxemia with few organisms present. Infection also produced by mouth. The glands in the neck are swollen and a general invasion of the body develops or the organisms penetrate through the lymph-glands back of the intestine, which are swollen and hemorrhagic. In other instances the rats develop an aspiration pneumonia, with inflammatory foci in the lungs containing great numbers of plague bacilli. In nearly all instances plague bacilli may be found in the contents of the intestine. Frequently there is a primary bubo in the submaxillary glands from which general infection develops. Infection also follows feeding, but not regularly. In some experiments 21 per cent. wild rats (*Mus decumanus*) have been infected by this channel and 44 per cent. ship rats (*Mus rattus*), in other instances 68 per cent. wild rats. In general the organs of animals dead of plague are employed for feeding. McCoy found 27 per cent. of the wild rats of San Francisco resistant to infection by feeding.

Fatal disease also occurs from the nasal passage and from the ocular conjunctiva. Martini produced primary pneumonia in wild rats by inhalation. In addition to the acute type of plague a chronic form exists in rats (Kolle and Martini[29], Albrecht and Ghon). The lungs are indurated, the bronchial and submaxillary glands caseous, the material containing virulent plague bacilli. Nearly all rats are sensitive to plague, including *Mus rattus*, the house rat. *Mus decumanus*, the migratory rat, *Mus Alexandrinus*, the roof rat, *Mus rufescens*, the Indian red rat, and the woods rat of California.

*Guinea-pigs.*—Subcutaneous injection of small quantities produces fatal infection in two to three days with an exudate and edema about the site of inoculation, hemorrhagic infiltrated connective tissue, and swollen lymph-glands. The spleen is much enlarged and shows many minute tubercles like those of pseudotuberculosis. Yellow punctiform necrotic foci occur in the liver and lungs. Chronic disease may occur, the animals dying of marasmus and the organs being sterile. Infection can be produced cutaneously through the shaven skin, especially of the abdomen. The animals die in five to six days and at autopsy have a hemorrhagic edema at site of inoculation, the adjacent lymph-glands are swollen and hemorrhagic and the internal organs show the changes which occur in subcutaneous inoculation. Intraperitoneal injection leads to the death of the animal in twenty-four to thirty-six hours, the exudate containing many encapsulated plague bacilli. Infection by mouth is uncertain, but occurs in about half the cases (Kolle, six out of twelve animals fed). Infection may also be produced through the nasal mucosa and the ocular conjunctiva. Guinea-pigs may also develop a chronic disease, like that in rats.

*Mice* are less sensitive to plague infection than rats. Subcutaneous inoculation produces a sepsis, fatal in five to six days. About half the animals fed on plague material develop disease. Inhalation may produce plague pneumonia, but usually septicemia.

Other small animals sensitive to plague infection are ground squirrels, cliff squirrels, *Citellus grammurus* (McCoy[30]), ziesels, weasels, prairie dogs, tarbagans, rabbits, and chipmunks (McCoy).

In apes, *Macacus radiatus*, subcutaneous infection of $\frac{1}{4}$ oesa of a plague culture leads to death in three to four days with a primary bubo of the adjacent lymph-glands, a wide-spread edema at the site of inoculation and a swollen red spleen. Intraperitoneal injection is fatal in a short time. Infection by mouth is nearly always successful. Introduction of the material into the nose leads to plague pneumonia in a certain number of cases. In other apes, *Semnopithecus entellus*, subcutaneous injection of minute amounts leads to a rapidly fatal septicemia closely similar to the septicemia seen in man.

Dogs are almost insensitive to plague bacilli. Cats can be infected by feeding on plague material in about half the cases (Kolle). Hyenas and jackals are resistant to subcutaneous inoculation, but develop disease from intraperitoneal inoculation and occasionally from feeding.

Bats (*Vesperugo noctula*) are sensitive (Gosio), the Japanese bat (*Vesperugo abrarmus*) resistant (Ohwoda).

Large animals (swine, cattle, sheep, goats, and horses) are but slightly susceptible to plague.

Birds almost without exception immune as well as cold-blooded animals. Insects, fleas, flies, bedbugs, and ticks take in plague bacilli when fed upon plague material. Flies (*Musca domestica*) may sometimes die from infection (Nuttall). They may carry living plague organisms about on their feet (Albrecht and Ghon).

The principal species of animals in which plague occurs *naturally* is rats. Here the lesions are like those produced artificially by subcutaneous inoculation, swelling of glands (buboes), hemorrhages beneath the skin, granular liver containing small white or grayish flecks, an exudate in the pleura, swollen spleen, etc. Other species in which the infection has been introduced and kept alive under natural conditions are ground squirrels and prairie dogs (McCoy).

**Virulence.**—The virulence of plague bacilli varies greatly under both natural and artificial conditions. Cultures may retain virulence unchanged for long periods as in the strain of Albrecht and Ghon which after thirteen months' cultivation (eleven generations) without animal passage was still intensely virulent, killing guinea-pigs by hemorrhagic septicemia by a dose of 1/100,000 of an oesa. Another strain retained its original virulence fifteen months. In other instances the virulence of cultures is lost with great rapidity. No satisfactory explanation has ever been offered for this condition. Virulence is best tested on rats by subcutaneous or conjunctival inoculation, on guinea-pigs by rubbing the material on the shaven skin (cutaneous). Mice are poorly adapted for virulence tests because of their irregular resistance. The virulence of somewhat weak strains can be restored by animal passage, but not that of very weak strains. It is best maintained by frequent animal passage, by preservation of cultures at low temperatures. Bielolowsky states that hemolysin production goes parallel with virulence.

Under natural conditions the virulence of plague cultures varies greatly and at times typical organisms are encountered quite without action on animals.

*Toxins.*—Clinical evidence, great prostration, etc., suggests the formation of a toxin by the plague bacillus, especially the plague lesions without bacteremia described in the fetus by the German Commission. Filtrates of old cultures (ten days) are toxic in large doses, 5 c.c. for rabbits and apes. This action is to be attributed to an endotoxin liberated from the body of the organism. In the opinion of some authors (Markl[31]) the toxicity of plague cultures is also due in part to metabolic products in the cultures.

**Immunological.**—Animals may be actively immunized against plague by vaccines of various types, living virulent cultures and killed cultures, by filtrates from old cultures, by endotoxins (Besredka), by nucleoproteins (Lustig and Galeoti[32]) and by various combinations. Vaccines have been employed in many countries for control of plague, the results being on the whole favorable. The serum of immunized animals has protective properties and this immune serum has also been widely employed in the control of the disease. It contains agglutinins, precipitins, specific bacteriolytic or bactericidal substances, but no definite antitoxins.

Plague in man exists in two distinct forms, the bubonic transmitted from plague-infected rats by the fleas and the pneumonic which prevails in epidemic form in cold weather. The organisms in the two diseases are alike but it may be noted that cases of plague pneumonia in bubonic plague do not seem to give rise to the disease, at least in hot countries. An interesting suggestion has been made by McCoy and by Wherry that the plague bacillus by residence in the bodies of rats may assume properties by which it can produce bubonic plague while by residence in the ground squirrel it may assume properties by means of which it may produce pneumonic plague.

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### Bacterium tularense McCoy and Chapin

Discovered by McCoy and Chapin[1] in a disease of ground squirrels previously described by McCoy[2] and characterized by its resemblance to bubonic plague in rodents.

**Morphology.**—In smears from the organs of infected animals small round or rod-shaped organisms occur enclosed in a delicate capsule. The rods measure 0.2 $\mu$ in breadth by 0.3 to 0.7 $\mu$ in length, the capsule 0.3 to 0.5 $\mu$ in breadth by 0.4 to 1 $\mu$ in length. After death the organism rolls up into coccoid bodies. In cultures the organisms are regular and coccoid, from egg cultures measuring 0.5 to 0.6 $\mu$ in diameter. With the halo caused by the capsule, they measure 0.7 $\mu$ in breadth by 1 to 1.5 $\mu$ in length. Capsules are usually not demonstrable in cultures. The organisms involute rapidly in cultures, showing enlarged and irregular forms. Coccoid bodies also appear in coagulated egg-yolk and yolk ovomucoid cultures. Capsules stained only when the bacteria are mixed with serum.

**Stained** best by anilin water, Hoffman's violet or Victoria blue, also by ordinary gentian-violet and carbolfuchsin, badly by methylene-blue. The capsules can be demonstrated in films from the tissues by carbolfuchsin, not by methylene-blue.

**Cultivated** first from animals by McCoy and Chapin on Dorset's egg medium, transparent drop-like colonies appearing in three to five days. Cultivated first from man by Wherry and Lamb[3] on coagulated hens, egg-yolk which may be diluted with 20 per cent. water or milk (McCoy and Chapin). Growth aërobic. On egg-yolk medium colonies are very minute on the second or third day. Later they may be 1 to 3 mm. in size. They appear like minute drops of ovomucoid, viscous, emulsifying with difficulty in salt solution. Growth also on ovomucoid yolk medium used for the cultivation of amebæ.[4] Cultivated by Francis[5] on serum glucose agar (horse serum 5 per cent., glucose 1 per cent.), on glucose blood agar (rabbit's blood 5 per cent.), on blood agar (rabbit's blood 5 per cent.). All these media are better if a bit of the spleen of a healthy rabbit is added. Subsequently grown by Francis[6] on beef infusion agar containing 0.02 per cent. of an amino-acid like cystine, on beef infusion agar containing a piece of fresh sterile rabbit spleen and on Loeffler's blood-serum coagulated at 20° C.

*Destroyed* at 55° to 60° C. in five minutes (McCoy). Killed by equal quantities of 2 per cent. tricresol in two minutes.

The organisms do not pass a Berkefeld filter 5.

Cultures visible after three months at 15° C.

**Pathogenic Action.**—Several rodents are infected in nature, California ground squirrels (*Citellus beechyi* Richardson), Utah ground squirrels (*Citellus mollis*), rabbits of southern Indiana, and the jack rabbits of Utah (jack rabbits, snowshoe rabbits, and cottontail rabbits), but not domestic or tame rabbits. Parker, Spencer, and Francis[7] have recently reported the infection of the common wood tick of Montana (*Dermacentor Andersoni*) with this organism. The so-called glandular type of tick fever may possibly be tularemia.

The disease may be transmitted to the desert squirrel (*Ammospermophilus leucusus*), and to mice, rats, guinea-pigs, rabbits, monkeys, and gophers among small animals, cats, dogs, and pigeons being immune. Large animals like swine, calves and goats are not susceptible. Infection produced by cutaneous, subcutaneous, nasal and intraperitoneal inoculation and also by feeding virulent material. In squirrels the most marked lesions are caseous buboes, great enlargement and congestion of the spleen and caseous granules, pin-point to 1 mm. in size, in the spleen and liver. In Belgian hares injection into the ocular conjunctiva produces a purulent exudate with ulcers, followed by the death of the animal (Wherry and Lamb). Guinea-pigs are highly susceptible, dying in eight days from 0.000001 to 0.0000001 c.c. of an emulsion of the spleen or liver of an infected animal, $\frac{1}{2}$ gram to 10 c.c. NaCl (McCoy and Chapin). Guinea-pigs immune to *Bacillus pestis* are susceptible to *Bacterium tularense*. Rats can be infected by large doses. The lesions bear a superficial resemblance to the lesions in

plague, but are not the same (Councilman and Strong[8]). Infected animals die of a true septicemia. In guinea-pigs and squirrels the organisms are present in enormous numbers in the spleen, are less numerous in the liver, the heart's blood and in the buboes.

**Transmission.**—Infection by *Bacterium tularense* can be conveyed by a number of biting insects, *Chrysops discalis*, *Stomoxys calcitrans*, *Ceratophylus acutus*, *Cimex lectularius*, *Polyplax serratus*, and *Hæmodipsus ventricosus*. The first four of these bite man as well as rodents. Infection is apparently the most common through the bite of *Chrysops discalis*. The disease has been transmitted from infected rabbits to healthy rabbits by the bite of *Chrysops discalis* and by the rabbit louse, *Hæmodipsus ventricosus*, from infected to healthy mice by the bite of the bedbug, (*Cimex lectularius*) and of the mouse louse, *Polyplax serratus* (Francis and Lake[9]). In nature rabbits are infected by the bites of the rabbit tick, (*Hæmaphysalis leporis-palustris*) and by the rabbit louse (*Hæmodipsus ventricosus*). The mite (*Liponyssus Isabellinus*) is also infectious. Fresh and dried bedbug feces are likewise infectious and produce the disease in guinea-pigs when fed. Guinea-pigs do not acquire the disease from the bite of the insect.

*Infection* in *man* was first reported by Wherry and Lamb in two individuals suffering from ulcerative conjunctivitis and lymphadenitis, positive cultures of *Bacterium tularense* being obtained in special media. These individuals were apparently infected mechanically from diseased rabbits. Subsequently it was shown by Francis that "deer fly disease" or "deer fly fever" is also produced by this organism. In deer fly disease the onset is sudden after the bite of a fly on the exposed surface of the body and is followed by pain, fever, prostration, and swollen, tender lymph-glands which drain the bitten area. The glands may suppurate and require incision. The fever lasts three to six weeks and is followed by a slow convalescence. Deer fly disease occurs during the seasonal prevalence of the fly, *Chrysops discalis*, in an area where jack rabbits are dying from "tularense" infection. The disease is apparently conveyed from the carcasses of the infected rabbits to man by this fly. Deer fly disease or deer fly fever has now been named "tularemia" by Francis.[10] Twenty-four cases of tularemia have been reported from Millard County, Utah, in 1917, 1918, and 1920. One fatal case was reported by Francis in 1919. Lake[11] has reported a number of cases of tularemia in laboratory workers. The organism was not present in the blood, but agglutination and complement fixation tests were positive. All recovered. Minute quantities of material can produce infection in man. Agglutinins for the organism appear in the blood of infected individuals. Rabbit fever, a disease among market men who handle rabbits, is apparently also tularemia (Francis).

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## Tribe 7. Hemophileæ, Nov. Trib.

Minute parasitic forms growing only in presence of hemoglobin, ascitic fluid, or other body fluids.

### GENUS 9. HEMOPHILUS Com.

Minute rod-shaped cells, sometimes thread forming and pleomorphic, non-motile, without spores, strict parasites, growing best (or only) in the presence of hemoglobin, and in general requiring blood-serum or ascitic fluid. Gram-negative.

Type species *Hemophilus influenzæ* (Pfeiffer) Com.
" *hemolyticus* Stillman and Pritchett.
" *para-influenzæ* Rivers.
" *pertussis* (Bordet and Gengou).
" *conjunctivitidis* (Koch-Weeks).
" *meningitidis cerebrospinalis septicemiæ* Cohen.
" *canis* (Friedberger) Rivers.
" *pertussis* of Ependorf.

### Hemophilus influenzæ (Pfeiffer) Com.

Discovered in the epidemic of 1889–90 by Pfeiffer[1] and first cultivated on hemoglobin-containing media by Pfeiffer and Beck.[2] Described in full by Pfeiffer[3] in 1893 and named *Bacillus influenzæ.*

**Morphology.**—Minute bacteria, 0.2 to 0.3 $\mu$ in breadth by 1 to 1.5 $\mu$ in length, often arranged in pairs end to end so as to resemble diplococci. Long threads measuring 10 to 15 $\mu$ are common, usually interpreted as involution forms. On blood agar cultures eighteen to twenty-four hours old the organisms are about 0.3 by 1 to 3 $\mu$, with many longer forms, 7 to 10 $\mu$ in length. Grassberger[4] states that threads are common on horse serum while Delius and Kolle[5] found them in poorly growing cultures. The water of condensation on agar slants often shows threads (Scheller[6]). Some strains do not show the typical morphology, but tend to grow in long threads looking like a leptothrix. It is not entirely clear whether these strains should be regarded as influenza or as some associated variety. Influenza bacilli are present in the morning sputum, in influenza, in enormous numbers in characteristic clumps, often containing thousands, between the pus-cells. Their arrangement is compared by the German writers to the appearance of fish in a net. In mixed infections the number of influenza bacilli may be very small and their recognition difficult. In early stages the organisms are usually extracellular. In later stages they may be chiefly intracellular.

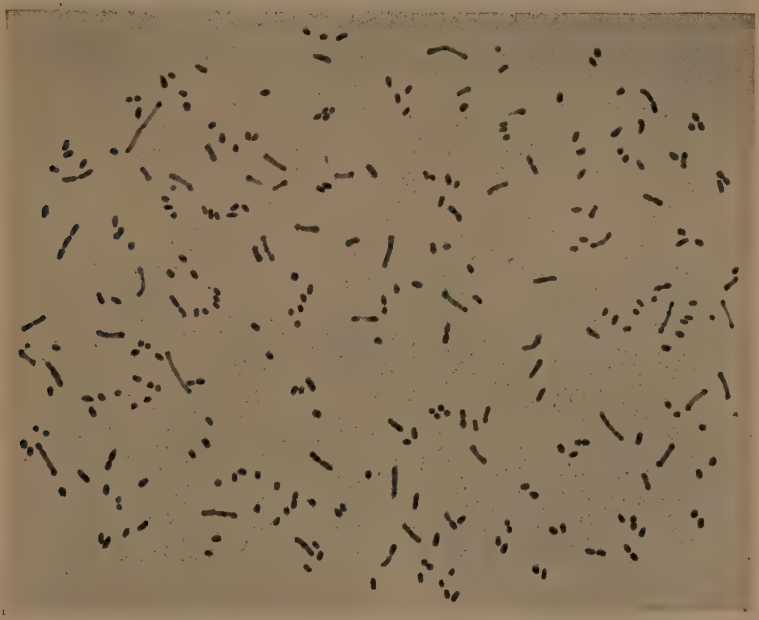


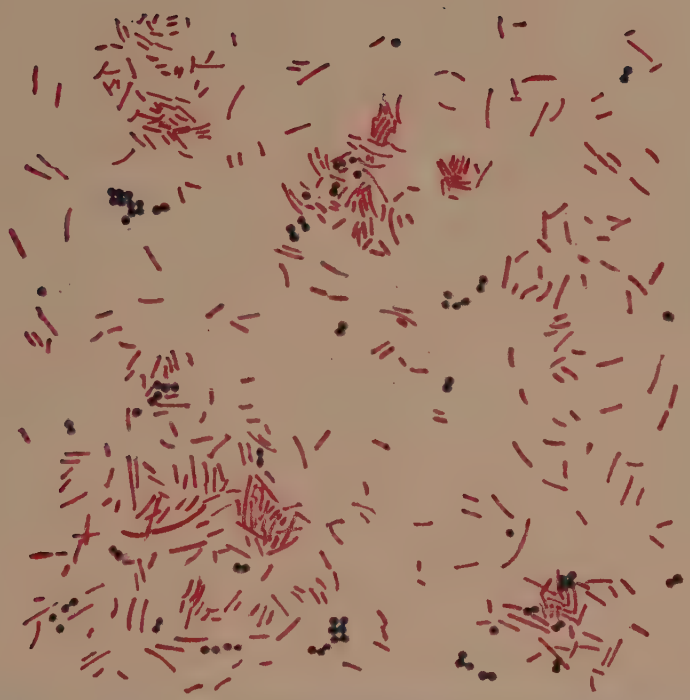
Fig. 120.—Influenza bacillus from blood agar twenty-four hours old, stained by Gram and carbolfuchsin.

**Staining Reactions.**—From the sputum best with 1 to 10 carbolfuchsin five to ten minutes or with Loeffler's methylene-blue two to five minutes. With fuchsin the organisms and the nuclei of the cells are sharply differentiated from the cell plasma. Also stains well with lightly acid gentian-violet, weak solutions of crystal violet (concentrated solution of dye to 20 parts water) or with safranin. In cultures stained easily with the ordinary anilin dyes, especially with fuchsin. With Loeffler's methylene-blue or Giemsa often shows polar granules. Gram-negative.

**Cultivated** originally by Pfeiffer on media containing human or rabbit's blood. Growth best at 37° C. Growth occurs at temperatures ranging from 22° to 37° C., seldom below 22° C. or above 41° C. (Park and Williams[7]). A number of special media have now been devised for the cultivation of this organism. It may also be cultivated in symbiosis with other organisms like the diphtheria bacillus. Highly aërobic, little growth in the depths of agar stabs. Scanty development in an atmosphere of hydrogen, microscopically only degenerate forms. Maximum growth at the end of eighteen to twenty-four hours. Growth more luxuriant in a closed system in which about $\frac{1}{10}$ the volume of air has been replaced by $CO_2$ (Zinsser[8]).

Fig. 121.—Influenza bacillus, aberrant forms, from blood agar twenty-four hours old, stained by Gram and carbolfuchsin.

Fig. 122.—Influenza bacillus, long threads from sodium oleate agar twenty-four hours old, stained by Gram.



Influenza bacillus and diplococci from sodium oleate agar, twenty-four hours old, stained by Gram.

*Blood Agar Plates.*—Colonies appear in eighteen to twenty-four hours at 37° C., small, transparent, about the size of streptococcus colonies. Under low power thin, homogeneous, with entire edges, non-granular, looking a little like drops of dew. Growth most rapid on agar with pigeon's blood, then with human or rabbit's blood. Better results obtained by smearing blood over the surface of the agar than by making blood agar mixtures. According to Pfeiffer the hemoglobin from red blood-corpuscles is the ingredient essential for growth. Growth also on hemoglobin media heated to 70° C. and in Czaplewski's medium, pigeon's blood in large quantities of agar (Auerbach[9]).

*Blood Agar Slants.*—Growth usually as isolated colonies after eighteen to twenty-four hours. When the colonies are closely approximated they may coalesce and form larger colonies. When the water of condensation contains blood it may become turbid, otherwise it remains clear.

*Blood Broth.*—Growth abundant when a good deal of surface is exposed to the air, as in wide-bottomed flasks.

*Chocolate Agar.*—Fairly abundant moist expansion.

*Bordet's Pertussis Medium.*—Moderate expansion.

*Sodium Oleate Medium* of Avery.—Rather abundant spreading growth.

*Milk* slightly acid in forty-eight hours or alkaline, the alkalinity not as marked as with *Bacillus pertussis* (Rivers[10]).

*Symbiosis.*—Growth on hemoglobin-free media in association with *Staphylococcus aureus* (Grassberger), gonococcus, and diphtheria bacillus (Cantani[11]). The other organisms can be killed by heating to 60° C. for three hours and still give positive results. Park and Williams state, however, that the pyogenic cocci tend to inhibit growth. Dead cultures of a variety of organisms, *Bacillus coli*, *Bacillus prodigiosus*, *Bacillus violaceus* and *Vibrio Metchnikovi* added to hemoglobin-free media give an abundant growth of the influenza bacillus. Neisser[12] has shown that the organism can be cultivated for many generations on ordinary agar with the Xerosis bacillus, but never acquires the capacity to grow on such media without it. Growth also in broth with Xerosis bacillus.

Jordan[13] reports that many strains of the influenza bacillus produce indol and reduce nitrates to nitrites. This has been confirmed by Rivers[14] who finds that some strains fail to give these reactions.

**Resistance.**—Very sensitive to drying, a pure culture diluted with water and dried twenty-four hours being destroyed with certainty (Park and Williams and Krumwiede). Vitality retained in dried sputum up to two weeks. Alive in blood broth cultures kept at 20° C. after several weeks. Destroyed in five minutes at 60° C. by boiling one minute. Very sensitive to ordinary disinfectants. Cultures kept at 37° C. remain alive only two to three days. At 22° C. five to ten days.

**Accessory Growth Factors.**—As originally pointed out by Pfeiffer the hemoglobin of the blood enables the influenza bacillus to grow on ordinary media. It has subsequently been shown that hematin represents the essential constituent. Recently it has been pointed out by Rivers[14] that two factors are necessary for the development of this organism, an autoclave-stable factor, hematin, and an autoclave-labile factor, which may be obtained from brewer's yeast grown on malt agar with a pH 5 to 6, washed in physiological salt solution three times, taken up in five to ten volumes of distilled water, boiled, and filtered.

**Pathogenic Action.**—The influenza bacillus possesses little virulence for ordinary animals, the original cultures of Pfeiffer, and Pfeiffer and Beck having no pathogenic action on subcutaneous inoculation. With rabbits Pfeiffer produced an acute death by intravenous inoculation, the animals showing dyspnea and a subnormal temperature. At autopsy practically no lesions were found beyond a small spleen and a hyperemia of the lungs. Cultures from the blood and organs were sterile. Organisms killed by chloroform were also fatal to rabbits in exactly the same way, the animals dying evidently of toxemia. Since then Kolle and Delius have shown that intraperitoneal injection of guinea-pigs, rabbits, and mice is frequently fatal, this result also being attributed to a toxemia. Similar results were obtained by Cantani on young guinea-pigs, small doses killing them by intraperitoneal inoculation, large doses being required for fatal results from subcutaneous administration. Perez,[15] however, by smearing the mouth cavity, nose, pharynx, and larynx of rabbits with the influenza bacillus produced areas of local inflammation from which influenza bacilli were cultivated. After injuring the bone and joints and then injecting influenza bacilli in the blood-stream, Perez also succeeded in producing foci of inflammation in the injured areas. In the same way abscesses were produced in the middle ear, brain, and peritoneum as well as pericarditis. After subdural inoculation Wollstein[16] has found meningitis in apes.

Attempts to produce influenza in animals by the influenza bacillus have been usually fruitless. Cecil and Steffen[17] have shown that by intratracheal insufflation in apes an inflammation of the bronchi and lungs may be caused which closely resembles the pathological picture of influenza in man. Cecil has also produced a reaction to the influenza bacillus in human volunteers which resembled a very mild influenza attack, without fever however.

*Toxins.*—The early experiments in which influenza bacilli were fatal to animals in a short time, but could not be obtained from the blood and organs after death, suggested the

presence of some kind of toxic substance in it. By heating cultures to 60° C. Cantani obtained an "endotoxin" which, when injected into the brains of guinea-pigs, produced the same lesions as followed intraperitoneal inoculation of living cultures. This "endotoxin" was also obtained by Slatineanu,[18] who cultivated the influenza bacillus on pigeon blood agar, washed it off with physiological salt solution, centrifugalized and took up the sediment with a mixture of fresh horse serum and physiological salt solution in such quantities that about 10 c.c. of this mixture corresponded to 0.25 cg. of dried bacterial substance. Of this so-called endotoxin $\frac{1}{2^5}$ c.c. killed guinea-pigs by intracerebral injection in six to ten hours with the same collapse of temperature as shown by intraperitoneal injection of living organisms. Intraperitoneal injection of 5 c.c. killed guinea-pigs in four to five days with enormous swelling of the spleen and a fatty degeneration of the liver. More recently Parker[19] in Zinsser's laboratory has found powerful poisons in young influenza cultures. The best poisons were found in broth cultures containing 5 to 10 per cent. defibrinated rabbit's blood, the broth having a pH of 7.8. These poisons were also produced in the filtrate from this blood broth (chocolate broth). They were toxic to rabbits in doses of 1.5 c.c., the animals showing marked prostration and muscular weakness, and dying in two to six hours in a considerable proportion of cases.

The incubation period was short, forty-five minutes to one and a half hours. According to Zinsser these poisons from the influenza bacillus are like the poisons obtained by Zinsser, Parker, and Kuttner[20] from streptococcus and typhoid filtrates, described by them as "X" substances.

As a result of all this experimentation it is evident that under ordinary circumstances the influenza bacillus is not pathogenic for animals. If the tissues be injured mechanically various types of lesions may be produced which are like the lesions in man. The nearest approach to the condition of influenza is the type of disease produced in apes by Cecil and Steffen.

**Distribution.**—In times of epidemic influenza, the influenza bacillus of Pfeiffer is widely distributed. It occurs in the sputum of the majority of the cases, the number of isolations depending somewhat on the skill of the bacteriologists who examine the patients. It is also frequently encountered in the sputum of individuals with febrile respiratory disease when the disease is not prevailing in epidemic form. It was found by Pfeiffer in tuberculous processes and may be present in bronchiectatic cavities. According to Boggs it may be present in cavity fluids without apparently giving rise to symptoms. It occurs in the respiratory passages in diphtheria (Jaehle[21]), in chronic laryngitis and tonsilitis (Wynekoop[22]), in bronchopneumonia in children (Wollstein[23]). It is rarely present in the blood during life (Madison[24]). Influenza bacilli are also isolated from many infections of the accessory nasal passages, the antrum of Highmore and the frontal sinuses, sometimes alone and sometimes in association with other organisms, pneumococci and streptococci. At autopsy on cases dying during epidemics of influenza the influenza bacillus is present in the pneumonic areas, sometimes alone but usually associated with other organisms like the pneumococcus or hemolytic streptococci. It is present in the meninges in influenza meningitis (Wollstein), sometimes in association with the streptococcus (Zinsser). It is sometimes, but not regularly present in the heart's blood. Influenza bacilli are also present at autopsy on individuals dead of diseases other than influenza. Thus Jaehle found them in the heart's blood in two fatal cases of scarlet fever and in fifteen cases of measles, in nine cases of chickenpox and occasionally in whooping-cough, while Wollstein has found them in bronchopneumonia and lobar pneumonia in children. They are frequently present in the lungs in fatal cases of diphtheria. After infection with the influenza bacillus individuals may carry the organism for some time as shown by Pritchett and Stillmann[25] and by Opie.[26] Occasionally the influenza bacilli cannot be found in outspoken cases of influenza by most careful investigators. Such cases were described by MacCallum[27] in autopsies on individuals dead of the disease in the pandemic of 1918. Such findings coupled with the failure to demonstrate the presence of immune reactions in the blood with the influenza bacillus of Pfeiffer have suggested that the disease is due to some type of filterable virus, the influenza bacilli playing the rôle of a secondary invader. Such a filterable virus has not thus far been demonstrated with any degree of certainty.

1. Pfeiffer: Deutsche med. Wchnschr., 1892, xviii, 28.
2. Pfeiffer and Beck: Deutsche med. Wchnschr., 1893, xix, 816.
3. Pfeiffer: Ztschr. f. Hyg., 1893, xiii, 357.
4. Grassberger: Centralbl. f. Bakteriol. [etc.], 1895, xxiii, 353.
5. Delius and Kolle: Ztschr. f. Hyg., 1897, xxiv, 327.
6. Scheller: Die Gruppe der hæmoglobinophilen Bakterien. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, v, 1257.
7. Park, Williams, and Krumwiede: Pathogenic Microörganisms. 1920, p. 455.
8. Zinsser: Textbook of Bacteriology. 1922, p. 482.
9. Auerbach: Ztschr. f. Hyg., 1904, xlvii, 259.
10. Rivers: Johns Hopkins Hosp. Bull., 1922, xxxiii, 149.
11. Cantani: Ztschr. f. Hyg., 1901, xxxvi, 29.
12. Neisser: Deutsche med. Wchnschr., 1903, xxix, 462.
13. Jordan: J. Am. M. Ass., 1919, cxxii, 1542.

14. Rivers: Johns Hopkins Hosp. Bull., 1920, xxxi, 50.
15. Perez: Deutsche Ztschr. f. Chir., 1902, lxiii, 502.
16. Wollstein: J. Exper. Med., 1905, vii, 335. *Ibid.*, 1906, viii, 681.
17. Cecil and Steffen: J. Infect. Dis., 1921, xxviii, 201.
18. Slatineanu: Centralbl. f. Bakteriol. [etc.], 1906, xli, 185.
19. Parker: J. Am. M. Ass., 1919, lxxii, 476.
20. Zinsser, Parker, and Kuttner: Proc. Soc. Exper. Biol. and Med., 1920, xviii, 49.
21. Jaehle: Ztschr. f. Heilk., 1901, xxii, 190.
22. Wynekoop: J. Am. Med. Ass., 1903, xl. 574.
23. Wollstein: J. Exper. M., 1905, vii, 335.
24. Madison: Am. J. M. Sc., 1910, cxxxix, 527.
25. Pritchett and Stillman: J. Exper. M., 1919, xxix, 259.
26. Opie, *et al*: Surgeon-General's Report., J. Am. M. Assoc., 1919, lxxii, 168.
27. MacCallum: Johns Hopkins Hosp. Rep., 1921, xx, Fase ii, 149.

### HEMOPHILUS HEMOLYTICUS Pritchett and Stillman

Pritchett and Stillman[1] have described strains of influenza bacilli which produce hemolysis on blood agar plates and also form amylase. To this organism Bergey[2] has given the name *Hemophilus hemolyticus*. It does not differ in other particulars from the usual influenza bacilli.

1. Pritchett and Stillman: J. Exper. M., 1919, xxix, 259.
2. Bergey: p. 269.

### HEMOPHILUS PARA-INFLUENZÆ Rivers

Described by Rivers as an influenza-like organism which requires only the autoclave labile-substance as an accessory food factor, not the autoclave-stable factor. The colonies resembled the colonies of the influenza bacillus, old colonies having a brownish granular center, very old ones having crinkled surfaces. They were Gram-negative, did not hemolyze blood, did not produce indol, reduced nitrates to nitrites and brought about no change in milk. One of Rivers' isolations showed a questionable acidity in dextrose, saccharose, and maltose.

Rivers: Johns Hopkins Hosp. Bull., 1922, xxxiii, 429.

### BACILLUS PERTUSSIS Bordet and Gengou

Cultivated from the sputum of pertussis patients by Bordet and Gengou.[1] Agglutinated by the patient's serum with which the compliment deviation reaction is also given. Usually regarded as the cause of pertussis.

**Morphology.**—In the sputum, and especially in the early stages of the disease, when it is present in large quantities, a small ovoid bacillus almost coccoid, somewhat thicker and longer than the influenza bacillus. The organisms are usually extracellular. Later they become more and more difficult to find in the sputum, and are usually intracellular. Single or in twos end to end. After long-continued cultivation on blood agar the organisms become smaller. In fluid media they retain their size. They show no particular tendency to pleomorphism, but threads appear in old cultures and in the water of condensation.

**Staining Reactions.**—Stained with difficulty, best with Kühne's methylene-blue or toluidin blue. Usually shows bipolar granules which may differentiate them from influenza bacilli, but this is not absolutely diagnostic.

*Gram-negative.*

**Cultural Characters.**—Cultivated first by Bordet and Gengou on a medium made of agar blood and glycerin potato extract, later by Shiga[2] on a similar medium containing horse blood instead of rabbit's blood, by Inaba[3] with goat's blood, by Klimenko[4] with dog's blood. Growth is slow, barely visible in twenty-four to forty-eight hours, often requiring microscopical examination for evidence of multiplication. On the third day the colonies are white, elevated, and circumscribed. In later generations the growth is more rapid and in many recently isolated strains more rapid development is evident than was first described by Bordet and Gengou. The colonies are usually whiter and thicker than the bluish and transparent colonies of the influenza bacillus and the growth more abundant when once established. On blood agar slants the growth is usually thicker than that of influenza, but does not spread widely, having

Fig. 123.—Pertussis bacillus, from twelve days' growth on blood agar, stained with gentian-violet.

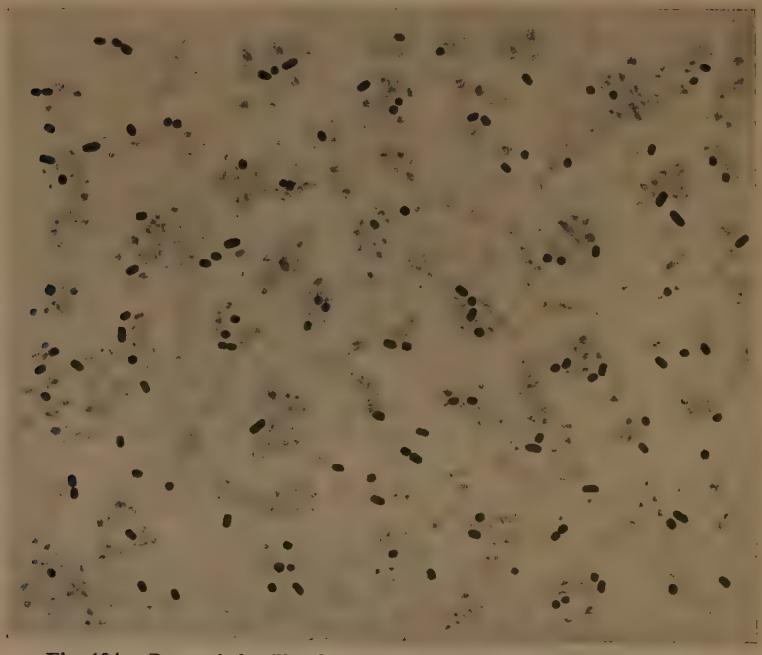


Fig. 124.—Pertussis bacillus from culture showing short coccoid forms.

more or less abrupt upright edges. The blood was never turned black in the original cultures of Bordet and Gengou, but later strains possess this characteristic. The blood is hemolized and blood agar media become clear. Poor growth on Pfeiffer's pigeon blood agar.

Cultivated also in broth containing 1 per cent. glycerin, 1 per cent. peptone, mixed with equal parts of rabbit blood, rabbit serum, or horse serum. The medium becomes turbid without much sediment when rabbit serum is used. Horse serum agglutinates the organisms and thus produces a viscid sediment of bacteria, the fluid above remaining clear. With immune horse serum this is more marked, the sediment showing abnormal forms and long threads.

On blood media the pertussis bacillus survives one to one and a half months; on agar it survives only ten to fourteen days (Klimenko).

The pertussis bacillus is differentiated with considerable difficulty from the influenza bacillus. Park, Williams, and Krumwiede state that it will grow on glycerin ascites agar and on plain agar, characteristics lacking in the influenza bacillus. Rivers[5] has also shown that *Bacillus pertussis* can be grown on plain media after a period of artificial cultivation, forms no indol and no nitrites, and makes milk very alkaline.

**Pathogenic Action.**—Bordet and Gengou have found *Bacillus pertussis* pathogenic to guinea-pigs by intraperitoneal inoculation of large quantities of bacteria from blood agar cultures (2 mg.). The animals die in twenty-four to forty-eight hours without increase of the organisms, *i. e.*, from toxemia. At autopsy these animals show hyperemia and petechiæ on the peritoneal surfaces, exudates in the pleura and pericardium, and fatty degeneration of the liver. Subcutaneous injection is also fatal, an extensive edema developing at the site of inoculation. With cultures killed with toluol or by heating to 56° C. the same changes were found at autopsy. Injected directly into the rabbit's eye the pertussis bacillus produces conjunctivitis with a corneal turbidity. Introduced into the bronchi it causes an intense necrosis of the bronchial mucosa.

The pathogenic action of the pertussis bacillus is usually attributed to an *endotoxin* (Bordet and Gengou). This may be obtained by Besredka's method of cultivating the organisms in agar containing horse's blood, glycerin water, potato extract, and veal broth. The three-day culture is taken up in 0.6 per cent. salt solution, dried "in vacuo" at 37° C., or over caustic soda. The residue is now rubbed up to a fine powder, taken up in water and kept twenty-four hours in the ice-box. This extract is now centrifugalized, giving a clear, slightly opalescent supernatant fluid of which $\frac{1}{4}$ to $\frac{1}{2}$ c.c. kills guinea-pigs with the same changes as are produced by the living organism. Rabbits are killed in twenty-four hours with 1 to 2 c.c. injected intravenously. At autopsy they show hemorrhages in the adrenals, hyperemia of liver and kidney, hemorrhages in liver, and pigmentation of connective tissue and kidneys. After subcutaneous injection of guinea-pigs a hemorrhagic edema and necrosis develop at site of inoculation.

Various attempts have been made to produce in animals a condition simulating pertussis in man. Klimenko has found that the direct injection of pertussis bacilli into apes and dogs produced a kind of pertussis with cough, the expectorations containing the bacilli. Carl Fraenkel[6] also found a peculiar bellowing cough in apes resembling whooping-cough in man, and a similar condition was produced in apes by Inaba. Interesting as are these observations the conclusion that the pertussis bacillus of Bordet and Gengou is the etiological factor in whooping-cough rests chiefly upon the agglutination of the organism by the patient's serum and the positive fixation of complement. The observations of Bordet and Gengou in this respect have not always been confirmed.

1. Bordet and Gengou: Ann. de l'Inst. Pasteur, 1906, xx, 731. *Ibid.*, 1907, xxi, 720. *Ibid.*, 1909, xxiii, 415.
2. Shiga, Imai, and Eguchi: Centralbl. f. Bakteriol. [etc.], 1913, lxix, 104.
3. Inaba: Ztschr. f. Kinderh., 1912, iv.
4. Klimenko: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1908, xlviii, 218. *Ibid.*, 1909, xlviii, 64. Deutsche. med. Wchnschr., 1908, xxxiv, 2, 2030.
5. Rivers: Johns Hopkins Hosp. Bull., 1920, xxxi, 50.
6. Fraenkel: München. med. Wchnschr., 1908, lv, 2, 1683.

### HEMOPHILUS CONJUNCTIVITIDIS (Koch-Weeks)

This organism, known usually as the Koch-Weeks bacillus was first observed by Koch[1] in "Egyptian eye disease" and described as small bacilli like those of mouse septicemia. The organisms were present in the catarrhal form of conjunctivitis, while in the purulent variety Koch found diplococci which he regarded as probably gonococci. Subsequently similar organisms were found by Weeks[2] in sporadic cases of conjunctivitis in New York and cultivated on 0.5 per cent. agar, but always in association with club-shaped organisms, probably the xerosis bacillus. Found also by Kartulis in Alexandria and obtained in mixed cultures. Obtained in pure cultures in 1890 by Weeks, who found them in over 1000 cases. Somewhat later cultivated by Morax.[3]

**Morphology.**—During the height of the disease and also in chronic cases the organisms are found in great numbers in and between the leukocytes, especially the polymorphonuclear neutrophils. When outside the cells they are single or in bunches. Usually longer and thinner than influenza bacilli, measuring 0.5 to 1 $\mu$ in length, and sometimes 2 $\mu$. Polar staining occasionally seen. Ends rounded. Organisms extremely thin or narrow. In cultures they grow longer, often as somewhat thicker long threads with thick ends, apparently involution forms.

*Stains* best with dilute carbolfuchsin (ten minutes), Nicolle's carbolfuchsin, Loeffler's methylene-blue. The organisms usually take the stains, but little especially safranin when used as a counterstain. Gram-negative.

**Cultivated** only at 37° C. On ordinary media it grows for only one or two generations, best on moist 0.5 per cent. peptone agar, not too alkaline. Cultivated on special media by Morax on Wertheim's serum agar, by Hofmann[4] on Wassermann's swine serum, nutrose agar, and on a mixture of two parts of slightly alkaline glycerin peptone agar and one part ascitic fluid to which sterile sheep or human blood was added in the proportion of 1 to 2. Grown by Kamen[5] and Markus[6] on Pfeiffer's blood agar made with human blood. It also grows in serum broth. In blood media human blood must be employed and not pigeon's blood. After twenty-four to forty-eight hours the colonies are moist, transparent, glistening points or small drops. Under low power they resemble small air-bubbles. They have round edges and lie on the surface from which they are easily lifted. Their resemblance to colonies of the influenza bacillus is striking, but they tend less to coalesce (zur Nedden) and with higher magnification (eighty times) are finely granular. When separated the colonies become larger, have a slightly wavy contour, and are more granular and opaque. Occasionally they are yellowish. In serum broth they produce a diffuse turbidity which gradually sinks to the bottom.

The cultures die out in about five days. At 20° C. they fail to develop, but if kept moist can be transferred up to sixty hours. Destroyed at 60° C. in one to two minutes. Not destroyed at 7° C. Destroyed by sun's rays in two and one-half hours at 43° C.

No pathogenic action on animals. The organisms have been planted repeatedly on the human conjunctiva, where they grow rapidly and produce characteristic conjunctivitis. (Morax, Weeks, etc.).

Recent observations by Knorr[7] suggest that the organisms found in Eygptian conjunctivitis are identical with the influenza bacillus and require the same accessory nutritive material in the media on which they grow.

1. Koch: Arb. a. d. K. Gsndhtsamte, 1887, iii, Aulagen, 62.
2. Weeks: Arch. f. Augenheilk., 1887, xvii, 318. New York Eye and Ear Infirmary Report, 1895.
3. Morax: Sur l'étiologie des Conjunctivitis aiguës. Thèse de Paris, 1894. Ann. d'ocul., 1903, cxxix, 156.
4. Hofmann: Ztschr. f. Hyg., 1900, xxxiii, 109.
5. Kamen: Centralbl. f. Bakteriol. [etc.], 1899, xxv, 401.
6. Markus: München. med. Wchnschr., 1901, xlviii, 2, 2137. *See also* Axenfeld. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl., 1913, vi, 545.
7. Knorr: Centralbl. f. Bakteriol. [etc.], 1924, xcii, 371.

### Bacillus meningitidis cerebrospinalis septicemiæ Cohen

Described by Cohen in 1909 from Bordet's laboratory. Found in 3 cases of septicemic meningitis. Like influenza, but more pathogenic.

Virulent to rabbits by intravenous inoculation, producing a rapidly fatal septicemia. Cultivated from the heart's blood. Subcutaneous inoculation fatal to rabbits in eight to fourteen days, producing a viscid purulent exudate at site of inoculation. At autopsy these animals show an extensive fibrinopurulent clot in the abdominal cavity with fibrinopurulent pleurisy, pericarditis, bronchopneumonia. The organism was cultivated generally from the lesions. Similar results obtained by trickling the organism into the nose of rabbits. Rabbits were immunized with this organism and an immune serum obtained which protected guinea-pigs and rabbits against infection. (This organism raises the question of the rôle of the influenza bacillus in meningitis.)

Cohen: Ann. de l'Inst. Pasteur., 1909, xxiii, 273.

### Hemophilus canis (Friedberger) Rivers

Obtained originally by Friedberger[1] and described as *Bacillus hemoglobinophilus canis*. Differentiated from the influenza bacillus by Odaira.[2] Isolated recently from the preputial secretions of six dogs by Rivers.[3] Smears

showed small Gram-negative organisms which appeared as the predominating form in cultures. They are pleomorphic and resemble the influenza bacillus.

**Cultivated** on a number of special media. On 2 per cent. rabbit blood meat-infusion agar young colonies are non-hemolytic, round, with a small granular area on top. These look like colonies of *Bacillus influenzæ*, but older colonies become more opaque. Old cultures show a luxuriant opaque growth resembling that of *Bacillus pertussis* rather than that of *Bacillus influenzæ*. Diffuse growth in liquid media. Good growth on 5 per cent. human blood agar in contrast to influenza which does not grow well on fresh unheated blood (Rivers[4]). This organism is further differentiated from *Bacillus influenzæ* since it requires the addition of only the autoclave-stable accessory food factor to media and not the autoclave-labile, the other organism requiring both (Rivers and Poole[5]).

Acid production without gas from glucose, fructose, galactose, mannitol, sucrose, and xylose, in the special media described by Rivers and Kohn.[6] No acid from maltose, lactose, dextrin, arabinose, and glycerol.

No change in milk. Indol positive. Nitrates reduced to nitrites. No hemolysis in solid or liquid media containing blood.

Vitality not great on certain media. The organisms do not live more than one or two generations on meat-infusion agar, meat-infusion agar and ascitic fluid, or 2 per cent. peptone agar and yeast extract. Successful transplants indefinitely on meat infusion agar and hematin or 2 per cent. peptone agar and hematin.

Non-pathogenic to white mice, guinea-pigs, and rabbits.

1. Friedberger: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1903, xxxiii, Orig., 401.
2. Odaira: Centralbl. f. Bakteriol. [etc.), 1. Abt., 1912, lxi, Orig., 289.
3. Rivers: J. Bact., 1922, vii, 579.
4. Rivers: Johns Hopkins Hosp. Bull., 1919, xxx, 129.
5. Rivers and Poole: Johns Hopkins Hosp. Bull., 1921, xxxii, 202.
6. Rivers and Kohn: J. Exper. M., 1921, xxxiv, 477.

### HEMOPHILUS PERTUSSIS of Ependorf

Described by Jochmann and Krause[1] in pertussis. Later cultivated from this disease in the convulsive stages by Jochmann and Moltrecht[2] in 42 cases and found in the areas of bronchopneumonia 23 times out of a total of 25 autopsies. Not identical with the influenza bacillus which it resembles, or with the pertussis bacillus of Bordet and Gengou.

1. Jochmann and Krause: Ztschr. f. Hyg., 1901, xxvi, 193.
2. Jochmann and Moltrecht: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1903, xxxiv, Orig., 15.

## GENUS 10. PHOTOBACTERIUM Beijerinck

Micrococci, bacteria, or vibrios producing a luminescence on natural objects, food, water, etc. Cultivated, as a rule, readily on artificial media where the luminescence may be lost. Common in sea-water. Originally described by Cohn.

Type species *Photobacterium phosphoreum* (Cohn).
" *Giardi* (Kruse).
" *Pflügeri* (Ludwig) Beijerinck.
" *phosphorescens* Beijerinck.
" *Fischeri* Beijerinck.
" *Balticum* Beijerinck.
" *indicum* (Kruse) Beijerinck.
" *cyano-phosphorescens* (Katz).
" *luminosum* Beijerinck.
" *Dunbari* (Migula).

### PHOTOBACTERIUM PHOSPHOREUM (Cohn)

Originally described by Cohn. Same as *Micrococcus lucens* v. Tieghem. The organism described by Ludwig as *Micrococcus Pflügeri* may be the same, but this is not clearly established. Beijerinck's organism, *Photobacterium*

*Pflügeri*, cannot be the same since Beijerinck had a small actively motile bacillus. Beijerinck's *Photobacterium phosphorescens* resembles this species in morphology (sarcina form), but is an actively motile fermenting organism.

Found on cooked fish, crabs, meat, etc., on which it produces whitish luminescence. Very large cocci growing best on dead sea-fish or in gelatin which is made from sea-water fish broth or which is rich in NaCl. No liquefaction of gelatin. Power of producing light gradually lost in cultures.

Cohn: Briefliche Mitteilung an J. Penn, abgedruckt in Verzameling van stukken betreffende het geneeskundig staatstoezigt in Nederland. Jaarg, 1878, p. 126.

### PHOTOBACTERIUM GIARDI (Kruse)

First reported by Giardi[1] and described as *Bacillus phosphorescens* Giardi by Kruse.[2]

**Morphology.**—Short bacilli, almost cocci, about 1 $\mu$ in diameter, in pairs about 2 $\mu$ in diameter or in chains of four. Like *Bacterium phosphorescens* (Fischer) Migula. Pure cultures produce luminescence in crustacea, the animals dying after three to six days. During life the infected individuals give off a greenish-white light. After death the cadavers are luminescent for a few hours and then take on a characteristic brown color. After several months' artificial cultivation the cultures lose both luminescence and virulence, regaining them after passage through fishes.

1. Giardi: Compt. rend. de la Soc. de biol., 1890, ii, 9. s., 188. [Abstr.], Centralbl. f. Bakteriol. [etc.], 1889, vi, 645. *Ibid.*, 1890, viii, 177.
2. Kruse: *In:* Die Mikroorganismen (Flügge). 1896, ii, 333.

### PHOTOBACTERIUM PFLÜGERI (Ludwig) Beijerinck

Regarded by Beijerinck[1] as the most luminescent of all the organisms in this group, but as somewhat rarer than *Photobacterium phosphorescens* (*Bacterium phosphorescens* Fischer) (Migula).

Elements small and long in gelatin made with peptone, showing less tendency to the formation of sarcina forms than does *Photobacterium phosphorescens*. The cells contain two well-marked spots or flecks which may be nuclei. Motility active in sea-water. Ferments glucose, levulose, and gelactose, but not maltose. Later described by Beijerinck as varying from small rods measuring 0.5 x 1 $\mu$ to spherical cells 0.5 to 2 $\mu$ in diameter. Swollen in presence of glucose. From Beijerinck's description this organism cannot be the same as the species described by Pflüger, Lassar, and Ludwig, who were working with a true micrococcus. According to Kruse, this species is the same as *Bacillus phosphorescens gellidus* described by Forster.[2]

1. Beijerinck: Centralbl. f. Bakteriol. [etc.], 1890, viii, 616. Referat by Ludwig.
2. Forster: Centralbl. f. Bakteriol. [etc.], 1887, ii, 337.

### PHOTOBACTERIUM PHOSPHORESCENS Beijerinck

First obtained by Fischer[1] from luminous fishes in the fish market in Kiel and in the Berlin aquarium. Regarded by Fischer as identical with the organisms first found by Pflüger and later described by Lassar and Ludwig (*Micrococcus phosphoreus*). Named by Beijerinck. Migula states that this species cannot be identical with the organisms described by Pflüger, Lassar, and Ludwig, since the latter workers had a true diplococcus. Beijerinck[2] says that this species shows a tendency to form sarcina masses. The individual elements contain two dark spots which may be nuclei. They are actively motile in sea-water, ferment glucose, levulose, and galactose, producing equal quantities of hydrogen and carbon dioxid, and have no action on maltose. This species is more common than *Photobacterium Pflügeri* Beijerinck, but not so powerfully luminescent.

**Morphology.**—Short thick bacilli with rounded ends, about one-eighth of a red blood-corpuscle in length and slightly less in width. The organisms appear often as micrococci. They are

single, in pairs end to end, or zoöglœa masses. Threads seldom formed. Close resemblance in morphology to *Bacillus prodigiosus*. Later described by Beijerinck[3] as varying from small rods measuring 0.5 x 1 $\mu$ to spherical elements 0.5 to 2 $\mu$ in diameter. Swollen in presence of glucose.

**Cultural Characters.**—*Gelatin Plates.*—Gray, white drops, under low power round and granular. Young colonies pale gray. Older colonies develop a dirty-brown color and concentric markings. No liquefaction.

*Gelatin Stab.*—Slow development along the line of inoculation, rapid superficial growth in form of a thin, gray-white expansion. No liquefaction.

Excellent growth on raw, cooked salt and fresh water fishes, on raw and cooked beef, sheep- and pig-meat. No growth on cooked potatoes, rice infusion, in urine, in acid or alkaline milk, or in broth without NaCl. Growth good only with free access of atmospheric oxygen and on moist media. Development at 10° to 15° C.

Cultures give off an intense green color which may rest on oxidative processes. Colonies in the depths of gelatin give off light as well as those on the surface. Optimum temperature for luminescence 15° to 24° C. Little luminescence at 25° C., none at 32° C. Some at 10° C. Cultures of the organisms added to sea-water produce luminescence as well as in 3 per cent. NaCl and Epsom salts, 1 per cent. magnesium chlorid, and fairly dilute sodium bicarbonate solution. In 3 per cent. NaCl solution luminescence is better than in ordinary sea-water.

No pathogenic action.

1. Fischer: Ztschr. f. Hyg., 1887, ii, 92.
———: Centralbl. f. Bakteriol. [etc.], 1890, viii, 617. Referat by Ludwig.
2. Beijerinck: Centralbl. f. Bakteriol. [etc.], 1890, vii, 338. Referat by Ludwig.

### PHOTOBACTERIUM FISCHERI Beijerinck

Isolated from the water in the harbor at Kiel and described by Fischer.[1] Named by Beijerinck.[2] Same as *Bacillus phosphorescens indigenus* Kruse.[3]

**Morphology.**—Short, thick rods, with thin rounded ends, measuring 1.3 to 2.1 $\mu$ in length and 0.4 to 0.7 $\mu$ in width. Slightly bent. Occasional spiral threads. Usually in pairs, joined at an obtuse angle like the cholera vibrio. Beijerinck[4] reports this organism as much smaller, measuring 0.1 to 0.3 $\mu$ in thickness and 1 $\mu$ in length.

*Motility* active.

**Staining Reactions.**—Stains by the usual anilin dyes.

**Cultural Characters.**—Growth on the usual gelatin medium after the addition of 3 per cent. NaCl. Growth good on gelatin made from infusion of herrings instead of infusion of beef. No growth on potato, milk, and broth or coagulated blood-serum. Excellent growth on cooked fish, which is covered in a few days with a gray-white, viscid mass, luminescent in the dark. Liquefaction of gelatin with a loss of substance from drying. An appearance as if the organisms actually ate a hole in the gelatin.

*Gelatin Plates.*—At the end of several days the colonies develop and show a depression of the gelatin in their immediate vicinity. At the end of about a week the deep colonies are generally round with sharp edges, producing a loss of substance in the gelatin and deepening to the bottom of the glass plate. The depression measures about 1 mm. in diameter and at the bottom the colonies lie as thin, dirty-yellowish disks. After several weeks the holes or depressions have a diameter of about 2 mm.; the colonies at the bottom are now more circular than disk form, having an appearance of small glass beads lying in the gelatin. Under low power the young colonies are round with sharp edges, pale sea-green in color, homogeneous, with isolated, slightly reddish granules or flakes. Older colonies are dirty, gray-yellow in color, made up of irregularly arranged flakes. In the neighborhood of the sunken colonies the gelatin acquires a reddish glimmer. The colonies often show radial striations.

*Gelatin Streak.*—Gradual development of a growth about 2 mm. in width and about 2 mm. deep, the bottom covered with a thin layer of yellowish material without any particular amount of fluid.

*Gelatin Stab.*—Gradual development of a small funnel-shaped liquefaction about 2 mm. wide at the end of a week, and about 1 cm. in depth, filled with air. Beneath this hollow space, which is often in the shape of a watch glass, lie flocculent crumbly masses of material containing relatively little fluid. In older cultures the hollow space increases in breadth and depth with gradual drying of the fluid material. Cultures several months old show a cone-shaped loss of substance, 3 to 5 mm. wide and 2 to 3 cm. deep, on the edge of which lie masses of culture material and at the bottom of which are thick deposits of this material without a trace of fluid.

This organism does not grow at room temperature, but develops at a temperature of 5° to 10° C. Under anaërobic conditions the development is slower, but does not cease. The cultures give off a bluish-white light without green, not so intense as that of *Photobacterium*

*indicum* (*B. phosphorescens indicus*). Luminescence depends upon the presence of atmospheric air. On plate cultures the colonies develop luminescence when they are on the surface. In stab cultures it occurs only in the upper part of the conical depression. Luminescence most powerful in fresh cultures, cultures two months old losing their power. It is destroyed by higher temperatures, about 35° C. in about five minutes and about 32° C. in a slightly longer time. The addition of salt favors the luminescence as well as the growth. The addition of cultures to sea-water renders it luminescent. The spectroscopic analysis of the light gives a continuous spectrum extending from Frauenhofer's line D to beyond G, the maximum intensity lying between E and the middle of F and G. No differences in the color could be noted.

Beijerinck finds that *Photobacterium Fisheri* liquefies gelatin rapidly, is sensitive to the action of cane-sugar, which in small quantities increases the luminescence, but in larger quantities destroys it. The motility resembles that of the vibrios. No fermentation in culture media. Best growth in fish infusions made with sea-water containing 1 per cent. glycerin, 8 per cent. gelatin, and 50 per cent. asparagin.

1. Fischer: Centralbl. f. Bakteriol. [etc.], 1888, iii, 105; 137.
2. Beijerinck: Referat by Ludwig. Centralbl. f. Bakteriol. [etc.], 1890, viii, 616.
3. Kruse: *In:* Die Mikroorganismen (Flügge). 1891, ii, 331.
4. Referat by Ludwig: Centralbl. f. Bakteriol. [etc.], 1890. vii, 338.

### PHOTOBACTERIUM BALTICUM Beijerinck

This organism was first isolated by Fischer in Kiel and described by Beijerinck who differentiated it from *Photobacterium Fischeri* by its slow liquefaction of gelatin, this being increased after cultivation. Luminescence is dependent upon the presence of peptone but independent of glycerin. Not sensitive to the action of cane-sugar, growth and luminescence good with 3 to 5 per cent. present. No fermentation. Motility like that of the vibrios. Best growth in fish infusion made with sea-water with 1 per cent. glycerin, 8 per cent. gelatin and 50 per cent. asparagin.

### PHOTOBACTERIUM INDICUM (Kruse) Beijerinck

First isolated from sea-water in the West Indies and described by Fischer[1] as *Bacillus phosphorescens*. Named by Beijerinck.[2] Same as *Bacillus phosphorescens indicus* Kruse.[3]

**Morphology.**—Small, thick rods with rounded ends about one-sixth to one-quarter a human red blood-corpuscle in length, and two to three times as long as broad. Organisms often slightly bent and attached to each other in pairs at an obtuse angle. Short threads usually more or less irregularly bent. Often in zoöglœa masses. On potato large, swollen, and irregular forms. No spores.

**Motility.**—Active in both the individual elements and in the threads which exhibit a serpent-like sinuous movement.

**Staining Reactions.**—Stains well with the ordinary anilin dyes. With methylene-blue marked polar staining like the members of the hemorrhagic septicemia group.

**Cultural Characters.**—*Gelatin Plates* (10 per cent.).—Development of colonies slow. They appear in thirty-six hours as very small, round, gray-white points just visible to the naked eye. Later the superficial colonies sink into the gelatin while the deep colonies liquefy the gelatin about them and break through to the surface. The colonies then appear as punctiform masses at the bottom of a sharply outlined semicircular depression. By the end of the fourth or fifth day the depression reaches the bottom of the glass. The gelatin then has an appearance as if it were gnawed into by small, slightly yellowish, round colonies lying in a kind of beaker whose edges are made up of dry gelatin. Under low magnification the small colonies are round with sharp edges, pale blue or sea-green in color, homogeneous, often showing a rosy shimmer. Larger colonies are round, circumscribed, pale blue-green, granular. Later the colonies may be brownish or dirty-yellow, granular, with well-marked radiating zones about a central mass.

*Stab Cultures.*—At the end of about four days a peculiar bowl-shaped depression filled with air about the size of a hemp-seed develops in the superficial part of the gelatin. From this along the line of inoculation a pale gray-white, thread-like turbidity extends into the gelatin with a small liquefied zone above. By the eighth to tenth day a bowl-shaped beaker-formed depression is produced by the loss of substance in the gelatin, on the bottom and sides of which lies a dirty-yellow, at times folded, membrane. The actual amount of liquid gelatin

is often so small that only a drop collects at the bottom of this beaker-like depression. The growth reaches the wall of the tube only after four to six weeks. The liquefaction is finally funnel-shaped.

*Agar Slant.*—Gray-white expansion luminescent in the dark.

*Broth.*—No growth in slightly alkaline 10 per cent. agar, made from meat infusion without peptone and salt. Excellent growth on the same medium after the addition of 2.5 per cent. NaCl. No growth in acid broth. Growth in slightly alkaline broth as a diffuse turbidity and scum with luminescence, especially after the addition of 0.5 to 3 per cent. NaCl.

*Coagulated Blood-serum.*—Excellent growth after several weeks as a gray-white, slimy indented mass along line of inoculation about ½ to 1 cm. wide.

*Potato.*—Slight development at 15° to 25° C. in two to three days as a broad, thin, white expansion which produces light only slightly. With potatoes made slightly alkaline by washing with soda solution the growth is somewhat better, but never very good. On potatoes contaminated by the "potato bacillus" or by moulds the organisms sometimes died out and sometimes developed better, due possibly to a change in the character of the potato.

*Rice Infusion.*—No growth.

*Bread Infusion.*—No growth.

*Cooked Fish.*—Growth excellent. At 25° C. at the end of thirty-six hours or at room temperature at the end of three to four days an abundant, gray-white mass covering the entire surface and beautifully luminescent in the dark. Abundant growth and luminescence on cooked fresh-water fish after immersion in sea-water or in 3 per cent. NaCl solution.

No development of luminescence on inoculation of cultures upon the surface of living fishes.

Good growth on raw and cooked meat of beef, calf, pork, sheep, geese, and pigeons, especially on the addition of NaCl.

*Milk.*—Usually no growth. After the addition of 0.1 to 0.5 per cent. sodium bicarbonate a slight development with a trace of luminescence.

Best development on slightly alkaline media, or on the acid superficial surfaces of fishes. No gas formed in media. No odor.

Growth only aërobic. Optimum temperature for growth and luminescence 20° to 30° C. Little growth at 10° C.

Destroyed by drying in thin layers in one-half hour and by a temperature of 55° C. in ten to fifteen minutes. Not destroyed by exposure to a temperature of −15° C. three hours or −14° C. six hours.

Luminescence bluish-white, often strong enough in cultures to enable one to tell the time by a watch. All media which permit development of the organisms show the reaction. Bacteria-free filtrates of fluid cultures entirely free from light, so the phenomenon rests definitely upon the presence of organisms. Both growth and luminescence depend upon the free access of the atmospheric air and upon sufficient moisture in the media.

**Non-pathogenic.**—Beijerinck reports that this organism, *Photobacterium indicum*, liquefies gelatin rapidly and completely like the spirilla and proteus forms. Luminescence powerful, maximum at 30° to 35° C. Luminescence increased by selecting highly active colonies and cultivating them continuously. Motility moderate and flexuous. Not especially sensitive to the presence of sugar, especially when asparagin is present which neutralizes the destructive action of the sugar. Luminescence in media containing asparagin and 4 per cent. glucose.

1. Fischer: Ztschr. f. Hyg., 1887, iii, 54.
2. Beijerinck: Mededeelingen der Koninklijke Akademie von Wetenschoppen. Afdeeling Natuurkunde 2 Reeks Deel VII. Amsterdam, 1890.
3. Kruse: *In:* Die Mikroorganismen (Flügge). 1896, ii, 330.

### PHOTOBACTERIUM CYANO-PHOSPHORESCENS (Katz)

First isolated from sea-water and described by Katz as *Bacillus cyano-phosphorescens*. Closely related to, if not identical with, *Photobacterium indicum* Beijerinck (*Bacillus phosphorescens* Fischer).

**Morphology.**—Rods with rounded ends from fresh agar cultures measuring 2.6 μ in length, this being about two and one-half times the width. Threads formed on potato. Often in pairs. In broth the threads may be 80 μ long bent and sinuous. No spores.

**Staining Reactions.**—Polar and lateral portions of the organisms stain more deeply than the center with methylene-blue and other anilin dyes. Gram-positive.

*Motility.*—Very active motility in twenty-four-hour broth cultures at 20° to 22° C. and in gelatin plates. Threads non-motile.

**Cultivation.**—*Grows* as an aërobe and facultative anaërobe.

No growth at low temperatures near freezing-point. Dies out at 32° to 34° C. Optimum temperature 26° C. Slow growth at 13° to 15° C.

Not destroyed by drying six hours at room temperature. Dies out in distilled water at room temperature in fourteen hours. Luminescence requires access of oxygen. In sea-water it is bluish with a touch of green, on fish cultures more greenish, on gelatin bluish. Light powerful enough to read large print in the dark. The reaction appears with the development of the organisms. It lasts several months, eight at a maximum.

Cultures poured into sea-water render it luminescent. Addition of salts (sodium chlorid and disodium phosphate) to media favor the reaction. Best medium slightly alkaline gelatin, with 0.5 to 2.7 per cent. NaCl.

*Agar Streak.*—Abundant growth, grayish-white, and viscid. After two to three weeks a development of secondary colonies, dome-shaped, ash-gray, with fat-like opalescence, often 3 mm. in size.

*Broth.*—Abundant diffuse turbidity, sediment, and rapidly formed superficial scum. The broth clears and becomes red-brown in color. No development in slightly alkaline rabbit muscle broth, without peptone or NaCl. No development in simple acid meat infusion nor in such infusion after neutralization. After the addition of 0.5 per cent. NaCl excellent growth. With 2.5 per cent. NaCl development much more abundant.

*Sterilized Fish Media.*—Rapid development at 20° to 24° C. Within forty-eight hours entire surface covered with a moist, thick, viscid, wax-like confluent mass or branched intertwined colonies. Moist and viscid consistency. Yellowish or yellowish-brown in six days on sea-carp. Growth on crab meat only where it is moist.

*Blood-serum or Coagulated Hydrothorax Fluid.*—Moderate development. Slow liquefaction.

*Cooked Eggs.*—Good growth with luminescence. Confluent viscid expansion, light brown on the egg-yolk, bright greenish-yellow on the egg-white.

No growth in plain milk. Development in milk containing NaCl. In milk containing disodium phosphate, scanty growth.

*Potato* made slightly alkaline by washing with disodium phosphate solution. Viscid, luminous gelatinous, confluent growth, reddish-brown in the middle, yellowish-gray at the edges.

No growth in slightly alkaline sterilized urine, rice infusion, cooked bananas, cocoa-milk, etc. Growth in cocoa-milk containing 1 per cent. NaCl or 1 per cent. NaCl and 1 per cent. peptone with turbidity like that in broth.

*Gelatin Plates.*—Six per cent. colonies appear in eighteen hours at 21° to 22° C. Surface colonies sharply outlined, round, measuring 2 to 3 mm., homogeneous, finely granular, light yellowish-green by transmitted light under low magnification. Deep colonies 0.25 to 0.4 mm. with sharply defined edges. Homogeneous and finely granular under low magnification. The contents of the deep colonies show active swarming movements at 17° C. and beginning liquefaction. In forty-eight hours the superficial colonies attain a diameter of about 6 mm., are irregularly fissured, dirty, brownish-yellow, and surrounded by a narrow zone of liquefied gelatin, 0.5 to 0.7 mm. The gelatin shows under low magnification a grayish-yellow color and granular masses. Deep colonies somewhat greenish, fissured, 0.3 to 0.5 mm. in diameter, surrounded by zones of liquid gelatin .05 to .1 mm. in width, light brown or light gray, with radial striations. With thickly seeded plates the gelatin may be completely liquefied in twenty-four hours. The plates give off a peculiar insipid odor, often noticed with other water bacteria.

*Gelatin Stab.*—Six per cent. surface liquefaction flat, or in the form of a watch-glass with funnel-shaped hollowing below, about 5 mm. wide in forty-eight hours. At the bottom of the liquid gelatin a grayish-white film develops from which radial extensions spread into the gelatin. After three to four days the superficial liquefaction attains a diameter of 18 mm. On the surface an intertwined grayish-white scum appears with grayish-white or yellow flocculent masses in the liquid gelatin. Eventually the entire tube of gelatin is liquefied. With 6 per cent. gelatin containing 2.7 per cent. NaCl growth much more abundant with more marked radial extensions into the gelatin. In 10 per cent. gelatin development somewhat slower. In 8 per cent. gelatin with 2 per cent. grape-sugar growth very slow. After more than fourteen days a small fluid column develops in the lower part of the line of inoculation, with flocculi in the liquid gelatin, a grayish-white flocculent precipitate, and a surface scum.

In peptone gelatin made from an infusion of fish without NaCl, the natural acid reaction being about 8 per cent., the development and liquefaction are much slower. On fish peptone gelatin made weakly alkaline with soda solution development and liquefaction like that in ordinary 6 per cent. gelatin. Liquefied gelatin reddish-brown.

Katz: Centralbl. f. Bakteriol. [etc.], 1891, ix, 159.

### PHOTOBACTERIUM LUMINOSUM Beijerinck

First found by Beijerinck[1] in the water of the North Sea. Same as *Bacillus luminosus* Kruse[2] and *Microspira luminosa* (Beijerinck) Migula.[3]

**Morphology.**—Long and short, actively motile, vibrios and spirilla which bend markedly during movement.

Grows only as an aërobe.

Causes rapid and complete liquefaction of gelatin with putrefactive products like those with cultures of proteus and putrefactive vibrios. This is due to a true extracellular enzyme.

Luminescence less than that of *Photobacterium indicum* (*Bacillus phosphorescens* Fischer), strongest at 15° C., but lasts a longer time and is weaker at lower temperatures. Disappears at 20° C. Very sensitive to small quantities of sugar. Glucose 1 per cent. stops luminescence. Three to 5 per cent. stops liquefaction. No growth with higher concentration.

1. Beijerinck: Arch. néerl. d. sc. exactes [etc.], 1889, xxiii, 104. Referat by Ludwig. Centralbl. f. Bakteriol. [etc.], 1890, vii, 339. *Ibid.*, 1890, viii, 616.
2. Kruse: *In:* Die Mikroorganismen (Flügge). 2. Aufl. 1896, ii, 331.
3. Migula: p. 1015.

### PHOTOBACTERIUM DUNBARI (Migula)

Found by Dunbar[1] in the water of the river Elbe and isolated on twenty different occasions. Known also as *Microspira Dunbari* Migula.[2]

**Morphology and Staining Reactions.**—Vibrios like the cholera vibrio in form and size, motility, and staining reactions.

**Cultural Reactions.**—Development more abundant than that of the cholera vibrio at 37° C. on agar and in peptone solution. Growth in gelatin plates and gelatin stab like that of the cholera vibrio. Reaction like the cholera-red reaction on the addition of sulphuric acid to peptone cultures.

Pathogenic to guinea-pigs which show symptoms like those produced by the cholera vibrio. Lethal dose about twice that of Koch's organism.

Luminescence found in these cultures by Kutscher[3] in broth, 10 per cent. gelatin, and on potato at about 22° C. Similar luminescent vibrios also found in the dejecta of a number of individuals.

1. Dunbar: Deutsche med. Wchnschr., 1893, xix, 799.
2. Migula: p. 1013.
3. Kutscher: Deutsche med. Wchnschr., 1893, xix, 1301.

### NON-SPORULATING ANAËROBES

Arranged in chronological order

*Bacillus diphtheriæ vitulorum* Loeffler.
" *ramosus* Veillon and Zuber.
" A Grigoroff.
" *fragilis* Veillon and Zuber.
" *furcosus* " "
" *serpens* " "
" *funduliformis* Hallé.
" *nebulosis* "
*Coccobacillus anaërobius perfœtens* Tissier.
*Bacillus mortiferus* Harris.
Norris' bacillus.
Voges' bacillus.
*Bacillus gracilis putidus* Tissier and Martelly.
*Coccobacillus præäcutus* Tissier.
" *oviformis* "
*Bacillus ventriosus* "
" *capillosus* "
" *multiformis* Distaso.
" *acuminatus* "
" *variabilis* "
" *pseudoramosus* Distaso.
" *bullosus* "
" *thetaiotaomicron* Distaso.
" *variegatus* Distaso.

*Bacillus cornutus* Distaso.
" *tenuis spatuliformis* Distaso.
" *tortuosus* Debono.
*Bacterium mucosum anaërobicum* Klinger.
" *melaninogenicum* Oliver and Wherry.
" *pneumosintes* Olitzky and Gates.
Holman's anaërobe.

### BACILLUS DIPHTHERIÆ VITULORUM Loeffler

Also known as Schmorl's bacillus, Bang's "Nekrose-bacillus," *Bacillus necrophorus*, *Actinomyces necrophorus*, *Streptothrix cuniculi*, etc.

This species was apparently first investigated by Loeffler[1] in the disease known as calf diphtheria, and named by him *Bacillus diphtheriæ vitulorum*. Somewhat later Schmorl[2] found thread-like organisms in the putrid liver of rabbits, and in an infection of these animals beginning on the lips and marked by tissue destruction and fibrinous inflammation of the serous surfaces, pleura, pericardium, and peritoneum. It was called *Streptothrix cuniculi* by Schmorl. Similar organisms were obtained by Flügge by the injection of syphilitic condylomata into rabbits and named by him *Bacillus necrophorus*. Apparently the same species or a closely related strain was described by Bang[3] as the "*Nekrosebacillus*," and by Macfadyen[4] in necrotic livers of beef and sheep. The species was named *Streptothrix necrophora* by Kitt[5] and *Bacillus necroseos* by Salmonsen.

This species or at least representatives of the group is regarded as the chief necrotizing agent in infections of many wild and domestic mammals and birds, including horses, cattle, sheep, swine, goats, antelopes, reindeer, kangaroos, dogs, rabbits, mice, apes, hens, and kites. The lesions are marked by necrosis and gangrene. They occur in the skin, about the hoofs, forming sloughs and fistulas (foot-rot of sheep), about the lips and gums, in the udders and sometimes in the eyes. Subcutaneous abscesses in many different animals are due to this organism. Ulcers and diphtheritic inflammation also occur on the genitalia, in the mucous membrane of the throat and the intestinal tract. Embolic foci are common, in the lungs, liver, spleen, kidney, and heart muscle.

The organism can produce infection from its own activities but is prone to invade injured and infected tissues, especially the skin and mucous membranes.

**Morphology.**—In old encapsulated foci the organisms appear as very thin short rods which are stained with difficulty. In fresh lesions they appear as bacilli or long threads. The long forms may have one end thicker than the other (Schmorl) and the bacilli may show true branching (Ernst[6]). This is not confirmed by Jensen.[7] The long threads are sometimes homogeneous and sometimes have coccoid or short cylindrical bodies in their protoplasm, separated at regular intervals by dark, finely granular masses. Other threads have a very granular protoplasm. Césari and Alleaux believe that the long filaments have a very thin envelope surrounding the protoplasm which is condensed into chromatic granules. The threads may be 10 to 20 $\mu$ in length and occasionally 50 to 100 $\mu$ or even more. In cultures the organisms appear as long thick threads, 0.75 to 1.5 $\mu$ broad and varying in length from a few micra to a hundred or more. Swollen and irregular forms are not uncommon. In old cultures the threads may fragment into short segments. No spores. Non-motile. Flagella not demonstrated.

**Staining Reactions.**—The organisms stain poorly with ordinary anilin dyes, showing unstained spore-like bodies in some threads and deeply stained round bodies in others. They stain well with carbolfuchsin and carbol thionin. The granulations are brought out by dilute Ziehl's stain and by carbol-thionin. Metachromatic granules may be demonstrated by Giemsa. They are Gram-negative, but retain the stain by Claudius' method. In sections the organisms are best found by hardening in Müller's fluid followed by alcohol, then staining by toluidin safranin, dehydrating in concentrated alcoholic safranin solution, decolorizing

in concentrated fluorescin in oil of cloves and finally counterstaining in aqueous methyl-green. The bacilli are stained red, the tissues green. According to Jensen no other organism can be stained by this method.

**Cultivated** with considerable difficulty, growing only as an obligate anaërobe between 30° and 40° C., best at about 35° C. Best cultures obtained in liquid media. Little or no growth on acid media. According to Césari and Alleaux it is best isolated in Martin's broth or in Truche and Cotoni's medium by adding traces of calcium sulphate. Colonies on blood-serum agar plates barely visible after forty-eight hours, pale white, round, sharply limited, under low power showing radially arranged prolongations (Schmorl). With high magnification these prolongations are seen to consist of a mass of filamentous threads.

Slant agar made with Martin's broth shows small isolated colonies with serrated edges, semitransparent and brownish (Césari and Alleaux). Stabs show grayish line growth, with irregular, thickened, lichenoid branches. Deep agar stabs at 37° C. give colonies in two to three days (Bang). They are bushy, 2 to 3 mm., in diameter, under low power appearing as masses of tangled threads.

Grayish expansion with sinuous borders and a wrinkled surface on coagulated serum slants (Césari and Alleaux). In stabs in serum, coagulated at the lowest possible temperature to retain its clarity, a branching line growth like the inverted fir-tree of *Bacillus anthracis* may occur.

Bang gives the growth in serum agar stabs as a whitish streak along the line of inoculation in the deeper layers, as a turbidity or as a limited zone of uneven bushy colonies. An abundant evolution of gas takes place.

Fair growth in 10 per cent. gelatin made from Martin's broth at 37° C. (Césari and Alleaux). Thick floccules develop which drop slowly to the bottom because of the viscosity of the medium. The gelatin is not attacked and solidifies on removal from the incubator. No growth on ordinary gelatin even if sugar or glycerin be added.

In serum broth a tangled mass of organisms develops at the bottom, the supernatant fluid remaining clear. Rarely a turbidity from a coagulation of the proteins (Schmorl).

Excellent development in Martin's broth boiled for ten minutes before inoculation with or without glucose (Césari and Alleaux). In ten to twenve hours a uniform turbidity occurs, with silky waves on shaking and several bubbles of gas. In twenty-four hours there is a dirty-white chalky deposit, adherent to the bottom of the tube. After three to four days the fluid clears. The odor is penetrating and very disagreeable. The organisms may be agglutinated. If glucose be present (0.2 per cent.) the turbidity is more marked, the gas more abundant and the reaction feebly acid. Some cultures do not attack the glucose.

Thick, cloudy floccules in liquid serum, floating in the serum and slowly dropping to the bottom. Serum not coagulated or digested (Césari and Alleaux).

Milk acidified and coagulated in four to six days.

No growth on potato.

Glucose and lactose usually fermented, although feebly.

No fermentation of saccharose or glycerin.

Indol positive. No hydrogen sulphid. Foul smelling gases develop in protein media and a fetid odor due to mercaptans.

**Resistance.**—The organisms are fairly resistant to deleterious influences. They live several months at ordinary temperatures in Martin's broth. In glucose Martin's broth they die out at 37° C. in a week. They are not definitely destroyed at 95° C. and have been found alive and virulent after drying eighteen weeks (Fr. Mayer[8]).

**Pathogenic Action.**—Virulent to mice, guinea-pigs, rabbits, and calves. Dogs, cats, pigeons, and hens are usually resistant. In mice, inoculation at the root of the tail is followed by a spreading necrosis which involves the muscles of the back and frequently passes through these into the peritoneal cavity. Subcutaneous inoculation in rabbits produces an area of inflammation which rapidly converts the tissue to a dry, caseous, necrotic mass. This tissue destruction spreads widely. Thus, inoculation on the ear leads rapidly to necrosis of the tissues about the head and neck, often involving the large blood-vessels. Thrombophlebitis may develop leading to embolic thrombi in the lungs, heart, etc. Intramuscular injection produces a rapidly progressive necrosis, intravenous inoculation embolic necrosis in the lungs with pneumonia and purulent pleurisy, also in the liver, brain, etc.

In guinea-pigs subcutaneous injection produces edema with necrosis and ulceration. The lesions may heal spontaneously. Intravenous injection of 1 c.c. quantities produces immediate death with loss of reflexes and dyspnea while $\frac{1}{2}$ c.c. causes death in fifteen minutes. Intraperitoneal inoculation of 1 to 2 c.c. quantities causes death in six to twelve hours with a thick yellow exudate in the peritoneal cavity. Occasionally subcutaneous injection in guinea-pigs produces an acute fibrinous pleurisy with death in eight to ten days (Cuillé).

In calves the organism may produce a typical diphtheritic inflammation.

In man small subcutaneous abscesses may arise from accidental infection.

*Toxins.*—Césari and Alleaux claim that this organism produces a toxin which gives rise to edema, necrosis, and ulceration in guinea-pigs on subcutaneous injection. Intraperitoneal injection has no effect. The material is destroyed by heating to 100° C. for five minutes.

*Note.*—Medium of Truche and Cotoni is of following composition: Peptone Chapoteaut 4 per cent., glucose 2 per cent., sea salt 5 per cent. Made alkaline up to a violet color by litmus.

1. Loeffler: Mittl. a. d. K. Gsndhtsamte, 1884, ii.
2. Schmorl: Deutsche Ztschr. f. Thiermed., 1891, xvii, 375.
3. Bang: Maanedsskrift for Dyrloeger Bd. II, 1890.
———: Baumgarten's Jahresber., 92, 314.
4. Macfadyen: J. Comp. Path. and Therap., 1891, iv, 143; 243.
5. Kitt: Bakterienjunde und path. Mikroskopie, 1889.
6. Ernst: Monatschr. f. prakt. Tierh., 1902.
7. Jensen: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, vi, 234.
8. Meyer: Multiple Nekrose der Leber. Diss., 1903.

### Bacillus ramosus Veillon and Zuber

Isolated by Veillon and Zuber[1] from multiple purulent osteo-arthritis in a child. The pus was very fetid and contained a small, very short, fine bacillus. The same organism was subsequently obtained from mastoiditis and pulmonary gangrene,[2] from appendicitis, and from dental caries (Monnier). Besson[3] regards it as a normal inhabitant of the intestine. In the opinion of Jungano and Distaso[4] *Bacillus ramosus* is the same as *Bacillus* A of Grigoroff and *Bacillus pseciloides* of Roger and Garnier.

**Morphology.**—Small slender bacilli in the tissues somewhat thicker than the organism of mouse septicemia. In cultures it occurs singly, in pairs joined at an acute angle like the letter V and in long filaments. It may show cylindrical or ovoid enlargements and irregular swollen contours like the diphtheria bacillus. Branching has been noted by Veillon and Zuber and by Guillemot.[5] Non-motile. No spores.

**Staining Reactions.**—The organisms stain feebly with the ordinary anilin dyes, gentian-violet and Loeffler's blue. They were described first as Gram-negative by Veillon and Zuber, subsequently as weakly Gram-positive.

**Cultivated** with difficulty as an obligate anaërobe, growing best at temperatures between 33° and 39° C. Grows well in an atmosphere of hydrogen. Agar colonies appear in the depths in forty-eight hours as very fine grayish points, under low power spherical or ovoid granular grayish masses with diffuse contours looking like bristles. Older colonies are grayish-yellow with undulating borders. Surface colonies are small, clear, transparent humid drops like colonies of the gonococcus, somewhat finer and more transparent than streptococcus colonies. Under low power they appear as small patches, grayish-brown, granular, and slightly cloudy. In sugar agar a small amount of gas is produced, but not enough to fragment the agar. Turbidity in sugar broth, with a faint whitish sediment and a small amount of gas which has a very characteristic fetid odor. Cloudy flocculent deposit in ordinary broth. Milk coagulated without digestion of the casein. No growth in gelatin. All cultures give off the peculiar, fetid odor.

Destroyed at 53° C. in thirty minutes. Organisms survive a month in cultures.

**Pathogenic** to mice, guinea-pigs, and rabbits. Subcutaneous injection gives rise to abscesses and the animals die in seven to eight days from cachexia.

1. Veillon and Zuber: Comp. rend. Soc. de biol., 1897, xlix, 253.
2. Veillon and Zuber: Arch. de. méd. expér. et d'nat., 1898, x, 517.
3. Besson: Practical Bacteriology, microbiology and serum therapy. Hutchens' translation, 1913, p. 571.
4. Jungano and Distaso: Les anaérobies. 1910, p. 150.
5. Guillemot: Recherches sur la gangréne pulmonaire. Thèse de Paris, 1899.

### Bacillus A of Grigoroff

Obtained by Grigoroff from pus of appendicitis.

Small rods like the diphtheria bacillus, straight or curved. Non-motile.
No spores.
Stains with ordinary anilin dyes. Gram-positive.
Grows only as a strict anaërobe, only in media containing sugars.

**Cultural Characters.**—*Glucose agar* colonies round or discoid on the second or third day at 37° C.

*Glucose gelatin* colonies appear on third day. Gelatin not liquefied.

*Glucose bouillon* shows a whitish deposit in forty-eight hours. Fluid remains clear, after a few days it becomes acid, and bubbles of gas collect along the sides of the tube. Deposit in flasks often characteristic in the form of large spherical masses resembling pearls.

No growth on *alkaline potato* in an atmosphere of hydrogen.

*Milk* acidified and coagulated from acid, not from ferment action.

Attacks glucose, lactose, maltose, levulose, sorbite, and rhamnose. No action on dulcite, mannite, and saccharose.

Non-pathogenic.

Grigoroff: Contribution a la pathogénie de l'appendicite. Thèse de Genève, 1905, p. 73.

### BACILLUS FRAGILIS Veillon and Zuber

Described originally by Veillon and Zuber from pus in appendicitis, subsequently by Grigoroff in appendicitis, by Cottet and Jungano in periurethral infections, by Guillemot in pulmonary gangrene, and by Monnier in dental caries.

**Morphology.**—Small short non-motile rods with rounded ends, either single or in pairs, looking like a diplococcus. It is thicker and longer in cultures than in the tissues and occasionally short swollen forms are present. No spores.

*Stains* feebly by gentian-violet and Loeffler's methylene-blue, best by dilute Ziehl. Gram-negative. Often has a granular appearance.

**Cultivated** only as a strict anaërobe, best at 37° C. The cultures have little vitality, and usually die out in seven to eight days on agar and in twenty to thirty days in gelatin.

*Surface agar colonies* very fine and transparent, grayish, slightly more definite than pneumococcus colonies which they resemble. After several days they become less visible and tend to be absorbed. *Deep agar colonies* appear in three to four days as small, round or slightly irregular, opaque ovoid points with smooth contours, yellow-brown in color. The colonies remain discrete and punctiform. Isolated colonies may be 1 mm. in diameter. *Gelatin colonies* at room temperature at the end of eight to ten days are small, punctiform, granular, with smooth borders, yellowish in color. No liquefaction. Abundant growth in *broth* with uniform turbidity and fine whitish deposit. More abundant growth in *glucose broth* without gas formation. Moderate growth in *glucose agar* with a small amount of gas, not enough to fragment the agar. All cultures give off a fetid odor.

The organisms are fragile and have little resistance to deleterious influences.

**Pathogenic** to guinea-pigs and rabbits. In guinea-pigs subcutaneous inoculation gives rise to abscesses and the animals die of cachexia in eight to thirty days. The organisms are much more virulent to rabbits. Subcutaneous injection produces abscesses and large phlegmons, the animals dying in seven to eight days. Intravenous inoculation produces a fatal cachexia, possibly from toxemia.

Veillon and Zuber: Arch. de méd. expér. et d'anat. path., 1898, x, 517.

### BACILLUS FURCOSUS Veillon and Zuber

Found by Veillon and Zuber in the pus from appendicitis. Rare.

**Morphology.**—In pus it appears as a small rod with two small branches at the end like a Y. In cultures the rods are longer and divide into two ramifications terminated by round or pyriform swellings. The branches are never very long and swollen extremities are numerous. The organisms are a little thicker than the tubercle bacillus.

Non-motile. Gram-negative.

**Cultivated** with difficulty. Growth slow and only at 37° C. *Surface agar colonies* appear in three to four days. They are very small, fine, gray points, slightly elevated, and look like pneumococcus colonies. They remain small and discrete. Under low power they appear as small yellowish masses with transparent borders, very finely granular. *Deep agar colonies* are so fine and transparent that they are difficult to see. Under low power they are fine, round, yellowish, with regular borders. They never become large, even when separated. *Broth* shows a fine precipitate with some gas, with a sour and very fetid odor. Cultures remain viable fifteen to twenty days.

**Pathogenic** to guinea-pigs. Subcutaneous inoculation produces abscesses and the animals die of cachexia after several weeks.

Veillon and Zuber: Arch. de méd. expér. et d'anat. path., 1898, x, 517.

### Bacillus serpens Veillon and Zuber

Isolated by Veillon and Zuber from the pus of mastoid abscesses and from pulmonary gangrene. The organisms found by Rist and Guillemot in gangrene of the lung and named *Bacillus radiiformis* are probably the same.

**Morphology.**—Fairly large rods with rounded ends. In cultures often arranged in pairs end-to-end and in pseudofilaments. Undulatory or vibratory motility in gelatin cultures (Guillemot).

Stains with basic anilin dyes and shows polar granules. Gram-negative.

**Cultivated** only as an obligate anaërobe, best at 37° C. Grows also at 20° to 27° C. Good growth in an atmosphere of hydrogen. Can be transferred after twenty to twenty-five days.

*Agar colonies* visible in the depths after twenty-four hours. They are small and round, under low power clear grayish granular masses of bristles, with sometimes a bunch of filaments at one of the poles. Later the colonies become more opaque with smooth borders. *Surface agar colonies* appear in forty-eight hours as small points. Later they form small very transparent but cloudy masses. With abundant inoculations they form a very fine surface expansion with discrete points like a growth of pneumococcus. Under low power the surface colonies appear like small, grayish, cloudy, but transparent masses. *Glucose agar colonies* resemble pneumococcus colonies. *Gelatin colonies* appear in five to six days as small, round, grayish masses which liquefy the gelatin slowly. The liquid gelatin remains clear and the colonies form small clouds suspended in the fluid. In *gelatin stabs* a grayish growth develops along the line of inoculation. The gelatin is liquefied, the fluid gelatin remaining clear, and flocculent masses falling slowly to the bottom and forming a white deposit. *Broth* is rapidly clouded and slowly clears with a whitish deposit. A fetid gas is evolved from all cultures, not enough to fragment agar.

**Pathogenic** to mice, guinea-pigs, and rabbits. Subcutaneous inoculation produces fetid abscesses and the animals die in seven to eight days.

Veillon and Zuber: Arch. de méd. exper. et d'anat, path., 1898, x, 517.

### Bacillus funduliformis Hallé

Found originally by Hallé in the vagina and in the pus in inflammation of Bartholin's glands. It is probably the same as the organism described by Rist and Guillemot as *Bacillus thetoides* from pulmonary gangrene, mastoid abscesses, etc. It has been obtained by Tissier in the stools of infants fed on a mixed diet composed chiefly of vegetables, and also in dogs.

**Morphology.**—Small curved rods with rounded ends. In cultures it is very pleomorphic, appearing as large swollen rods, curved tortuous branching filaments, or ball-like masses. In exudates it occurs within the pus-cells as disseminated individuals or in masses.

**Stained** with difficulty by ordinary anilin dyes. Gram-negative.

**Cultivated** only as an obligate anaërobe, growing best at 37° C. In sugar media it produces gas with a very fetid odor. In broth kept in a vacuum it produces only a slight turbidity, the broth clearing later. The odor is foul.

According to Tissier this species produces acid in dextrose, lactose, and saccharose, has no action on native proteins, but decomposes proteoses without giving indol. A small quantity of $H_2S$ is produced.

**Pathogenic** to guinea-pigs, subcutaneous inoculation producing gangrenous abscesses. Rabbits are not susceptible.

Hallé: Recherches sur la bactériologie du canal genital de la femme è l'état normal et pathologique. Paris, 1898.

### Bacillus nebulosis Hallé

Small organism like the bacillus of mouse septicemia. Usually straight or curved. Occasionally swollen at the center and tapering at the extremities. No involution forms. No spores. Gram-negative. Growth slow, only at 37° C. No gas produced from sugar media. Occasionally produces abscesses in rabbits and guinea-pigs on subcutaneous inoculation.

### COCCOBACILLUS ANAËROBIUS PERFŒTENS Tissier

Described by Tissier[1] as a small oval or elongated coccus, isolated or in pairs or short chains of three to four elements. Size 0.8 to 1 $\mu$. Gram-negative. Non-motile. Destroyed in fifteen minutes at 60° C. Growth only as an anaërobe at 37° C. No growth on the surface of *ordinary agar*, on *acid media*, or *media* containing *glucose* or *lactose*. Growth in agar with some water of condensation between the agar and glass as a fine powdery deposit.

Grows in *deep glucose agar* at the lower limit of the acid zone as a grayish-white turbidity, which under the microscope is found to be composed of innumerable fine regular colonies. Agar broken up by fine bubbles of gas. In the depths of the tube the colonies are somewhat larger, but not more than 1 mm. in diameter, lenticular. Gas not inflammable. It diffuses through the agar and is absorbed at the end of fifteen days. Rapid growth in *sugar broth*, with a sediment in twenty-four hours composed of very small grayish-white granules. Growth in *ordinary broth* slower. No growth in *milk*.

*Non-pathogenic.*

Differentiated from *Micrococcus fetidus* of Veillon,[2] which produces chains, is Gram-positive, gives gas but rarely, and forms clumps in broth. Differentiated from *Staphylococcus parvulus* of Veillon and Zuber,[3] which is a very small coccus in diplococci or in bunches, is decolorized by Gram, grows at 22° to 23° C., and produces characteristic granular brownish colonies in gelatin, granular colonies in agar, gives off a quantity of fetid gas, and is pathogenic for guinea-pigs and rabbits.

1. Tissier: Recherches sur la flore intestinale des nourrissons. Paris, 1900, 70.
2. Veillon: 1. c.
3. Veillon and Zuber: 1. c.

### BACILLUS ANAËROBIUS MINUTUS Tissier

Isolated from the stools of infants and described by Tissier.

**Morphology.**—In the stools the organisms occur as long rods, 2 to 4 $\mu$ in length, very thin, straight, with round ends, practically never curved. In fluid media they appear as delicate thin slender diplobacilli. In old cultures forms 6 to 8 $\mu$ long are found. On solid media the rods are small, 2 to 4 $\mu$ in length, in pairs and short chains of three, four to six elements. No bifurcated forms. Non-motile. No spores.

**Stains** well with ordinary anilin dyes. Gram-positive.

**Cultivated** only as an anaërobe at 37° C. Slow growth in *deep glucose agar* tubes. A turbidity develops in about six days in an anaërobic zone about 3 cm. below the surface. Under the microscope this appears as a multitude of fine, regular, round, or oval colonies. The colonies increase in size for three to four days, but remain fine, the size of needle points, whitish, and almost transparent. Older colonies are grayish and irregular, under low power embossed. The reaction of the agar becomes acid, but gas is not evolved. On *glucose agar slants* fine bluish transparent colonies appear in five to six days. In fluid media there is usually only a fine powdery deposit without turbidity. No growth in *milk*. *Non-pathogenic.*

*Note.*—This organism is regarded as different from *Bacillus fragilis* of Veillon and Zuber and from *Bacillus caducus* of Hallé.

Tissier: Recherches sur la flore intestinale des nourrissons. Paris, 1900, p. 81.

### BACILLUS MORTIFERUS Harris

Found in a liver abscess by Harris. No growth on ordinary laboratory media. Cultivation effected on media containing human blood or serum, placental blood, or hydrocele fluid.

**Morphology.**—Small non-motile bacilli with rounded ends varying in length and thickness, occurring singly, in pairs, and in short chains, resembling streptococci. No spores.

*Staining Reactions.*—Gram-negative. Easily stained by carbolfuchsin and gentian-violet.

**Cultural Reactions.**—*Hydrocele-fluid Agar Slant.*—Along line of surface inoculation, scattered, elevated, semiopaque, gray-white, smooth, round colonies, measuring 1 to 2 mm. in diameter.

*Hydrocele-fluid Agar Stab.*—Abundant, white, granular, translucent growth along line of inoculation with the production of gas-bubbles. Turbidity and precipitate in water of condensation.

*Hydrocele-fluid Glucose Agar Stab.*—Abundant development with vigorous gas formation. On the surface a heavy growth in the water of condensation. Vigorous, white, granular growth along line of inoculation with bubbles of gas.

*Hydrocele-fluid Broth.*—Uniform turbidity; no scum. An abundant, semiviscid, flocculent whitish sediment.

*Hydrocele-fluid Litmus Milk.*—Slight acid reaction and greenish-blue cream-ring. No coagulation. Gradual digestion of casein, the milk becoming almost transparent and dark purple-red in color.

*Hydrocele-fluid Gelatin.*—Small, round, white opaque colonies. No liquefaction.

*Human Blood Glucose Agar Stab.*—Abundant development with a moderate evolution of gas which has a disagreeable aromatic odor. Hemoglobin colored brown.

*Blood Broth.*—Uniform turbidity with gas formation and white semiviscid precipitate. Hemoglobin turned brown.

*Litmus Milk and Blood.*—Acid reaction. No coagulation. No peptonization. Hemoglobin color lost.

*Peptone Broth.*—Faint turbidity. White, semiflocculent, semiviscid precipitate. The hemoglobin retains its cherry-red color.

*Bacillus mortiferus* produces sulphids in dextrose-free broth and causes gas production in proteins without the addition of sugar. In Smith fermentation tubes of hydrocele fluid and dextrose-free broth of a reaction of 0.1 + gas is produced whose composition is roughly $\frac{H}{CO_2} = \frac{3}{1}$, that is, an excess of hydrogen over carbon dioxid, together with traces of a gaseous sulphid, probably hydrogen sulphid.

**Pathogenic Action.**—*Bacillus mortiferus* produces hepatic lesions which correspond closely with the lesions of the liver in the case from which it was isolated. On intravenous or subcutaneous inoculation of rabbits, guinea-pigs, and mice, the animals die in a few days; at autopsy the liver substance is studded with yellowish-white roundish masses, varying in size from 0.25 to 0.3 mm. and containing cheesy material. On microscopical examination the liver cells are destroyed in the centers of these masses, while the adjacent liver cells show hyalin necrosis. Occasionally there is an overgrowth of connective tissue as in intralobular cirrhosis in man.

Harris: J. Exper. M., 1901–05, vi, 519.

### Norris's Bacillus

This organism was isolated by Norris from the pus in a case of multiple liver abscesses.

**Morphology.**—Somewhat pleomorphic bacilli in the liver pus, staining faintly by usual methods. They were usually slender, of various lengths, but thicker, irregular forms were present which might be involution forms. They were best demonstrated by dilute carbol-fuchsin and were irregularly Gram-positive. In the liver tissue the organisms were best shown by Nicolle's method and by Sterling's gentian-violet. In cultures some organism showed curious involution forms of excessive size; others a bipolar staining, and others spore-like spaces. In later cultures these involution forms disappeared. Non-motile

**Methods of Isolation.**—Norris's bacillus grew originally in symbiosis with an anaërobic coccus. Pure cultures were finally obtained in deep tubes of neutral 1 per cent. glucose agar. The colonies developed only at 37° C. in the depths, 2.5 to 3 cm. below the surface. Exposure to the atmosphere promptly killed them. Good growth was obtained in sugar agar or gelatin with ascitic broth. No gas produced. Animal inoculation negative.

This organism was put by Norris in the group of anaërobic intestinal bacteria, along with the species described by Veillon and Zuber.

Norris: J. Med. Research, 1901–02, vi (n. s. i), 97.

### Voges' Anaërobe from Periarticular Phlegmon of Cattle

From the pus from characteristic cases of periarticular phlegmon Voges has obtained a very minute rod just on the limits of visability. It could be cultivated only anaërobically. In broth it produced a turbidity with considerable sediment. The broth had a stinking odor like that of pus. Minute grayish-white colonies developed in the upper part of agar stabs in two to three days at 37° C. Cultures kept alive only five to six generations. Killed at 50° C.

Pure cultures reproduced the disease, especially in young calves, the organisms invading the blood and organs.

Intraperitoneal inoculation of guinea-pigs produced a septicemia fatal in forty-eight hours.

Voges: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1902, xxxi, 138.

### Bacillus graciles putidus Tissier and Martelly

Found by Tissier and Martelly in decomposed meat.

Small thin bacilli, often in short chains. Non-motile. No spores. Destroyed at 100° C. Gram-negative.

**Cultural Reactions.**—A strict anaërobe producing small, grayish, lenticular colonies on *glucose agar* and somewhat irregular colonies on *plain agar*. No liquefaction of *gelatin*. Turbidity and sediment in *broth*. No change in *milk*. All cultures give off a putrid odor. Meat and fibrin are softened and disintegrated with an abundant evolution of gas. No acid from sugars. Produces a lipase saponifying fats. In cultures containing fibrin produces proteoses, amino-acetic, butyric, and valerianic acids, and a small quantity of $H_2S$. No indol or phenol. Produces a tryptic diastase.

Tissier and Martelly: Am. de l'Inst. Pasteur, 1902, xvi, 865.

### Coccobacillus preäcutus Tissier

Observed by Tissier in the stools of infants fed on a mixed diet. In the stools it appears as a coccobacillus with very fine and very pointed extremities in the form of a shuttle, resembling the spirilla of the mouth, sometimes isolated, sometimes in groups of 2 or 3, sometimes in long chains of eight to ten elements. Each individual is 5 to 10 $\mu$ long. In cultures it is pleomorphic and shows swollen forms with refractile points at one end. Motility very rapid and undulatory.

**Stains** by ordinary anilin dyes except by Gram's method.

**Cultivated** as a strict anaërobe growing at 37° C. and at 22° C. Lives in sugar media eight to ten days. In *deep sugar agar*, colonies in twenty-four to thirty-six hours are fine, transparent, very regular, lenticular, the largest 1 to 2 mm. in diameter. Abundant gas is evolved, fragmenting the medium rapidly. *Agar* becomes acid and does not give off odors. Growth good in *gelatin*, fine colonies without liquefaction. Turbidity and powdery deposit in *liquid media*. No coagulation of *milk*. No action on cooked white of egg. Acidity in glucose. No action on lactose or saccharose. No attack on proteoses. No indol. It decomposes cream and forms alkaline soaps.

*Non-pathogenic.*

It is distinguished from *Bacillus fusiformis* of Veillon and Zuber by its motility and greater vitality.

Tissier: Ann. d. l'Inst. Pasteur, 1908, xxii, 189.

### Coccobacillus oviformis Tissier

Found by Tissier in the stools of infants fed on a mixed diet or one rich in albuminoids.

In the stools it appears as a short rod or an elongated coccus, or more often as a diplococcus made up of oval elements resembling the ordinary form of the enterococcus. In liquid media it produces chains of five to six elements. Pleomorphic, non-motile.

**Cultivated** as a strict anaërobe growing at 37° and 22° C. Cultures live only five to six days. *Deep sugar agar* colonies lenticular, opaque, whitish, of variable size. The larger may be 2 to 3 mm. No gas. Growth in *gelatin* as fine colonies without liquefaction. In *liquid media* it produces slight turbidity with pulverulent sediment. No coagulation of *milk*. No action on *cooked white* of *egg*. Acidity in *glucose*. None in *saccharose* or *lactose*. Attacks peptones without producing indol.

*Non-pathogenic.*

Tissier: Ann. d. l'Inst. Pasteur, 1908, xxii, 189.

### BACILLUS VENTRIOSUS Tissier

Obtained rarely by Tissier in the stools of infants fed on a diet like that of adults. Also found in the stools of dogs.

In the stools it appears as a very fine rigid bacillus with square ends, isolated or in groups of two to three individuals. In solid media it gives long chains of forty to fifty very short elements. When the medium has less nutritive properties it swells up in the central part and looks like a small ball on a flower stand. It stains by ordinary basic anilin dyes. Gram-positive. The swollen areas retain the color strongly. Non-motile. No spores.

**Cultivated** only as a strict anaërobe at 37° C. Dies out in cultures in four to five days. In *deep agar* the colonies are fine, lenticular, regular with smooth borders, 2 to 3 mm. in diameter when well-developed. No gas. Growth in *gelatin* without peptonization. In *liquid media* it produces a slight turbidity and a pulverulent deposit. No coagulation of *milk*. Acidity in *glucose*. No acid in *lactose* or *saccharose*. No action on albuminoids. Attacks only proteoses. No indol.

*Non-pathogenic.*

Tissier: Ann. de l'Inst. Pasteur, 1908, xxii, 189.

### BACILLUS CAPILOSUS Tissier

Found by Tissier in two instances in the stools of infants fed on a mixed diet.

In the stools it appears as a large curved bacillus or as filaments more or less inflected. It looks like "perfringens," but differs in staining reactions. In solid media it appears as regular bacillary forms, isolated or in chains of two to three elements, as long tangled filaments like masses of hair. It also shows spirals thickened in the center with fine extremities, analogous to appearances seen in old cultures of *Bacillus acidophilus*. Non-motile. No spores.

**Stains** well by ordinary dyes. Gram-negative.

**Cultivated** as a strict anaërobe growing only at 37° C. Survives ten to fifteen days in cultures. In *sugar agar* it gives at the end of forty-eight hours fine, granular, irregular colonies with fine, regular, ray-like prolongations. No gas. It grows in *gelatin* at 37° C. without peptonizing it. The gelatin becomes solid on cooling. It develops badly in *liquid media* and gives only an insignificant turbidity. No coagulation of *milk*. Slight acid from *glucose*, none from *saccharose* or *lactose*. No action on non-hydrolyzed albuminoids. Acts on proteoses without producing indol.

*Non-pathogenic.*

Tissier: Ann. de l'Inst. Pasteur, 1908, xxii, 189.

### BACILLUS MULTIFORMIS Distaso

Isolated by Distaso from the stools of dogs.

Large bacillus, stubby or bent in very irregular forms. No spores in sugar agar. Motile. Gram-positive.

Grows in *deep sugar agar* as lenticular, opaque, very flat colonies. Large quantities of gas are produced and the agar is broken up into fragments. No characteristic odor.

*Glucose gelatin* dissolved and rendered turbid.

*Milk* coagulated after three to four days, the coagulum being slowly dissolved and clear spaces forming in the casein.

*White of egg* decomposed.

*Glucose* and *lactose* decomposed with a whitish powdery precipitate and a brownish pigment. Supernatant fluid does not clear. The culture has a very marked valerianic odor.

Saccharose not attacked.

Indol produced in traces.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1911, lix, 97.

### DIPLOBACILLUS ACUMINATUS Distaso

Frequently observed by Distaso in the stools of adult man. It appears in pairs with rounded ends, usually arranged like the diphtheria bacillus,

that is, in parallel rows. No chains. In young cultures it is small, but in old cultures it may become three times as long and appear as slender, often flexuous filaments. Often it appears as a V. Non-motile. Gram-positive.

In *sugar agar* the colonies are small, irregular, and translucent, about the size of grains of sand. The medium becomes turbid, but the turbidity is not due to acetic acid. No growth on ordinary *slant agar*, or in ordinary *gelatin* at 22° C. Grows well in deep *sugar gelatin* at 37° C., producing a feeble turbidity with floccules scattered along the column of gelatin which is not dissolved, and a whitish flocculent precipitate at the bottom of the tube. No gas production. *Milk* coagulated in twenty-four hours with a feeble odor of butyric acid. In this medium it gives filamentous and long rigid forms. It attacks *glucose*, *lactose*, and *saccharose*, giving a turbidity and an ill-defined odor. It grows very badly in *bouillon*, producing a turbidity and showing fine regular individuals microscopically.

No indol.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, 433.

### BACILLUS VARIABILIS Distaso

Described by Distaso from the dejecta.

Small coccobacillus with rounded ends, often swollen and curved at one end. No chains but long flexuous filaments. Encapsulated. Very slender in media. Non-motile. Gram-negative.

Grows only as a strict anaërobe. Does not survive more than ten days. In *deep sugar agar* it produces round transparent colonies with a moderate amount of gas which cracks the column of agar. No growth on *gelatin* at 22° C. or on *ordinary agar*. Grows well in *sugar gelatin* at 37° C. producing a turbidity and a whitish powdery deposit. *Gelatin* not dissolved. No action on *milk*. *Glucose*, *lactose*, and *saccharose* attacked slowly with gas and a feeble odor of butyric acid. These fluid media show no turbidity, but a whitish deposit at the bottom of the tube. The organisms in them are very slender. Ordinary *broth* rendered turbid with a marked odor of skatol. Indol positive. Abundant growth in *bouillon* containing white of egg which is not attacked. The medium becomes slightly turbid and gives a pronounced odor of skatol.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, Orig., 433.

### BACILLUS PSEUDORAMOSUS Distaso

Found by Distaso in the intestinal tract of adult man.

This organism resembles *Bacillus ramosus* of Veillon, appearing as small, delicate, flexuous bacilli often with sharp ends arranged at an angle. It also often forms small chains. Non-motile. Gram-positive.

Grows only as a strict anaërobe. Colonies round, opaque, about the size of a small pinhead. No growth at 22° C. or in ordinary gelatin at 37° C. Very feeble growth in sugar gelatin at 37° C.

Feeble attack on *glucose*, *saccharose*, and *lactose* with a slight odor of butyric acid. The media are at first rendered turbid, with a whitish deposit later at the bottom of the tube. Slow coagulation of *milk* requiring a month, a dark serum being expressed. Turbidity in *bouillon* containing white of egg. The white of egg does not become dark, but the culture gives an odor of skatol. Indol positive.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, Orig., 433.

### BACILLUS BULLOSUS Distaso

Obtained by Distaso from the intestinal contents.

Small rectangular bacillus staining intensely at both poles. In twenty-four-hour cultures also spherical or oblong forms occur which stain uniformly, and long slender rods with an enlargement in the middle or at one end. Sometimes the organism bifurcates at one end. Motile. Gram-negative.

**Cultivated** only as an anaërobe. Cultures survive six to seven days. Colonies in *deep sugar agar* like small grains of sand. Under the microscope they are round with a dark center and refractile borders. Growth in *milk* with a feeble acidity insufficient to coagulate. No attack on *gelatin* or on *white of egg*. *Glucose* attacked with a terminal acidity of 2.45 per cent.

estimated in $H_2SO_4$. No action on *lactose* or *saccharose*. Abundant gas formation. No indol.

Distaso considers that this bacillus represents a form intermediate between the "Tethoide" of Hallé and the bacillus which Ghon and Much have isolated in cases of influenza.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, Orig., 433.

### Bacillus thetaiotaomicron Distaso

Found frequently by Distaso in the dejecta.

Very pleomorphic organisms appearing as a coccus, in an elliptical form, in the shape of a kidney-bean or a bell-clapper. The elliptical forms stain only at the extremities with sometimes a transverse striation. The other forms stain uniformly. Motile. Gram-negative. Colonies in *sugar agar* are transparent, fairly large, round with clean edges and give rise to considerable bubbles of gas. *Milk* coagulated into a compact mass from which a dark serum is expressed. Growth in *gelatin* without peptonization. No attack on *white of egg*. Feeble action on *glucose* and *lactose*. Indol positive.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, Orig., 433.

### Bacillus variegatus Distaso

Found by Distaso in the intestinal contents.

Rods of different forms, sometimes short and regular, sometimes flexuous and filamentous extending across the entire field of the microscope. Ends sometimes rounded, sometimes pointed. The organisms are arranged in two's, sometimes in the form of a V, very rarely in chains. Motile. Retains Gram's stain in certain areas.

Colonies in *sugar agar* resemble grains of very fine sand, especially when they are separate. Under the microscope they are regular in form with clear refractile borders. Outside of these are other colonies which are embossed. No gas production. *Milk* coagulated after several days without expulsion of serum. No action on *gelatin* or *white of egg*. *Glucose* and *lactose* attacked feebly. Indol positive.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, Orig., 433.

### Bacillus cornutus Distaso

Reported by Distaso as constantly present in the dejecta.

Very small irregular bacilli occurring either as single elements or as elements disposed at an angle. Ends often curved. Closely resembles *Bacillus bifidus* of Tissier. In white of egg media it resembles the diphtheria bacillus. Non-motile. Gram-positive.

Colonies in *deep sugar agar* visible only after forty-eight hours, their growth being arrested within 3 cm. of the surface. Organisms survive about twelve days. No growth on *ordinary gelatin* at 22° C. Best medium *sugar gelatin* at 37° C. It forms flocculi in this medium which settle to the bottom of the tube while the medium clears. Attack very slow on glucose with gas production. No action on other sugars. No coagulation of *milk*. Feeble growth in ordinary *broth*. No indol.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, Orig., 433.

### Bacillus tenuis spatuliformis Distaso

Isolated by Distaso from the stools of dogs.

Bacilli with tapering ends, separated and arranged in parallel groups, sometimes straight, sometimes curved, often enlarged at one extremity in such a way as to have the shape of a spatula. No spores in sugar agar. Motile. Gram-positive.

Colonies in *deep glucose agar* are small, round, translucent. Some gas formation. *Glucose gelatin* is dissolved with a flocculent precipitate and clear supernatant fluid. *Milk* coagulated in two to three days, the coagulum being later dissolved. Small portions of the coagulum are left at the bottom of the tube in a turbid liquid. *White of egg* broken up into fragments. *Glucose* attacked feebly with a white precipitate and a black pigment. No action on *lactose* or *saccharose*.

Indol positive.

Distaso: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1911, lix, Orig., 97.

### BACILLUS TORTUOSUS Debono

Obtained from human feces by Debono.

In cultures it appears as straight rods which vary slightly in size; usually they are about the size of the diphtheria bacillus; sometimes they seem to assume a diphtheroid disposition and long chains are common, especially in agar and broth cultures. No spores. Non-motile. Gram-positive.

A strict anaërobe and difficult to cultivate.

In *deep glucose agar* growth ceases at 1 inch from the top. The colonies are small, irregular, grayish-white, and translucent. Under the microscope they are white, granular, with very irregular ill-defined margins. Very little gas formation. Medium clear. No growth on *plain gelatin*. On *sugar gelatin* at 37° C. it forms a white granular deposit at the bottom of the tube; at 22° C. the growth is extremely slow and scanty; in stab culture it forms a delicate line of growth along the track of the needle, white in color, with granules and small masses here and there; microscopically this growth is found to be made up of threads disposed parallel to each other, with tangled masses corresponding to the granules. No liquefaction of gelatin. Uniform turbidity in *broth*. No action on *white of egg*. *Milk* acidified without clotting. Acid and gas in small quantity from *glucose*, *laciose*, and *saccharose*.

Debono: Centralbl. f. Bakteriol. [etc.], 1 Abt., 1912, lxii, 229.

### COCCOBACTERIUM MUCOSUM ANAËROBICUM Klinger

Cultivated from a brain abscess by Klinger.

**Morphology.**—Cocci in the pus about 0.4 $\mu$ in diameter arranged as diplococci, smaller and larger masses. After several generations in pure cultures it assumes the form of short rods, 1 to $1\frac{1}{2}$ to 2 $\mu$ long. In sugar media swollen forms appear, oval, sausage-shaped, with well-stained polar bodies. Such organisms contain glycogen, stained brown with Lugol's solution. Chains frequent in broth.

**Stains** well with carbolfuchsin, gentian-violet, badly with Loeffler's methylene-blue. Decolorized by Gram. Probably encapsulated. Non-motile.

**Cultivated** only at 37° C. with serum added to the media, and only anaërobically. All cultures have an intense odor of cheese. Bacterial growth always viscous. Gas formation in media (gelatin) with or without sugar. Indol formed abundantly in sugar broth.

*Serum Agar.*—Small lentil-shaped, sharply outlined, isolated colonies in the depths of stabs. In the course of four to five days they are 4 mm. in diameter.

*Agar stab* shows the same type of colonies in the depths, irregular in size, lying with their largest diameter vertically.

*Serum Gelatin.*—Good growth as a viscid sediment. Complete liquefaction at 37° C.

*Milk* containing serum first coagulated and then peptonized. Little growth in plain milk.

*Serum-broth* with or without sugar shows turbidity and sediment.

*Coagulated beef serum* not liquefied.

Indol and $H_2S$ in all fluid media.

Subcutaneous injections kill mice, guinea-pigs, and rabbits in three to five days with spreading phlegmon. The pus is thin and has a very disagreeable odor. Little or no effect from intravenous or intraperitoneal injection.

Klinger: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxii, 186.

### BACTERIUM MELANINOGENICUM Oliver and Wherry

Described by Oliver and Wherry from anaërobic blood agar cultures from the throat, tonsils, infected surgical wounds of the abdomen, urine from suspected focal infection of the kidney, and from the feces in chronic dysentery following ameba infection.

**Morphology.**—Somewhat polymorphous rods about 0.8 $\mu$ by 1 to 3 $\mu$ in size. Non-motile.

**Staining Reactions.**—Gram-negative. Non-acid-fast.

**Cultivated** readily at 37° C. on +1 *human blood agar slants* made anaërobic by the pyrogallic acid method. In primary cultures the colonies are visible within one to two weeks. Pure subcultures yield a confluent black dry layer of growth within which lie somewhat polymorphous rods embedded in the masses of pigment. As growth increases the hemoglobin

throughout the agar slant disappears until finally the medium appears like a slant of plain agar. On *infusion agar* the growth is greatly facilitated by the presence of hemoglobin and pigment production is absolutely dependent on it. In *sodium phosphate broth* (+1) the organism produces acid from dextrose, levulose, lactose, saccharose, maltose, and mannite. Galactose is not attacked. No growth on *potato*, on *agar*, or in *broth* made from Liebig's meat extract with or without dextrose or maltose. Dense flocculent growth in *gelatin* containing pleuritic fluid at 37° C. No liquefaction. Fairly luxuriant, white, moist growth on Loeffler's *blood-serum*. *Litmus milk* slowly acidified, but not coagulated.

The rods themselves are non-pigmented. The pigment occurs as extracellular amorphous masses and apparently is a melanin. It is insoluble in alcohol, ether, chloroform, acetone, carbon disulphid, and carbon tetrachlorid. It slowly dissolves in sodium hydroxid solution.

Oliver and Wherry: J. Infect. Dis., 1921, xxviii, 341.

### Bacillus pneumosintes Olitsky and Gates

Discovered by Olitsky and Gates in the filtered nasopharyngeal secretions of patients in the early stages of influenza, also in the lungs of rabbits infected with nasopharyngeal secretions. Regarded by the authors as the etiological agent in epidemic influenza.

**Morphology.**—Minute non-motile organisms two or three times as long as broad, occurring as solitary cells, in twos or in chains of three to four elements. They are usually bacilloid, the long axis measuring 0.15 to 0.3 $\mu$. After long cultivation on artificial media the organisms are 0.5 to 1 $\mu$ long.

**Stained** best by methylene-blue. Gram-negative.

**Cultivated** originally in human ascitic fluid or ascitic fluid agar containing a piece of fresh sterile kidney tissue, either under a vaselin seal in a McIntosh and Fildes jar or under a covering of oil. The solid medium was composed of 1 part beef infusion, 2 per cent. agar pH 7.4 to 2 parts human ascitic fluid. A hazy growth was visible about the piece of kidney on the fifth day. Cultivated also in symbiosis with the influenza bacillus, the pneumococcus, streptococci and staphylococci.

The strains subsequently became adapted to artificial conditions and were cultivated on a variety of media under anaërobic conditions. The organisms require growth-stimulating substances which may be found in fresh tissue or body fluids, vegetable tissues like potato, turnip, or parsley, or the products of bacterial metabolism. The addition of dextrose enhances the value of the medium. Tiny gray colonies develop in the depths of solid media, revealing under high magnification dense irregular masses of bacteria with a fringe of single organisms. A diffuse cloud appears in fluid media in eight to sixteen hours and reaches its greatest density in three to five days. Blood agar plates, after seven to ten days, show very minute colonies, almost submicroscopic in size, round, or convex, raised, colorless, and translucent, with entire edges.

Growth best on media titrated to pH 7.4 on the Sörensen scale. Dextrose split to acid which inhibits the growth. Limiting pH = 5.2 to 5.3. *Killed* at 56° C. in one-half hour, by chloroform vapor in one and one-half hours. May be filtered through Berkefeld filters V and N.

**Pathogenic Action.**—Intratracheal injection of mass cultures in rabbits produces a rise in temperature, conjunctivitis, leukopenia, and a diminution of the mononuclear cells. At autopsy the lungs are edematous and emphysematous with discrete or diffuse hemorrhages on the surface. On section they show hemorrhagic edema and hemorrhages in the parenchyma. The trachea and bronchi have a mucopurulent exudate covering the exfoliated and hemorrhagic epithelium. Old strains are devoid of pathogenic action.

Olitsky and Gates: J. Exper. M., 1921, xxxiii, 713. *Ibid.*, 1922, xxxv, 813.

### Holman's anaërobe

Isolated by Holman and Krock[1] from the oral cavity of thirteen human beings, 3 rabbits, and 1 guinea-pig, in cooked meat media in which abundant gas production occurs in twenty-four hours. From this Veillon agar tubes are made and colonies fished in two to three days. The organisms are very small, well under 0.5 $\mu$ in length, and a little less in width. They are smaller than *B. prodigiosus*, *B. bronchisepticus*, and *B. influenzæ*, and about the size of *B. pneumosintes* of Olitsky and Gates. They are non-motile,

Gram-negative, occur singly, often in pairs, in irregular groups and are persistently anaërobic. In Veillon shake tubes the colonies never grow nearer than 1 cm. to the surface and are remarkably uniform in size in any particular tube. They vary from very tiny colonies when crowded to large disks or compound disks when widely separated. Surface colonies are raised, with a clearer sharp border and of a grayish-white color. In Veillon agar containing sugar, abundant gas may be produced or gas production may be absent. This is also true of fluid media in Holman's modification of Hall's anaërobic culture tube.[2] No acid produced. In liver-peptone broth it grows as fine granules with only a very slight cloud. Gas production in cooked meat medium is constant and abundant, the gas containing traces of $H_2S$.

This anaërobe passes through a Mandler filter (9), a Berkefeld N and a Berkefeld W, holding back *Bacillus pyocyaneus*. Cultures grown on Veillon agar slants in anaërobic jars do not pass the filters, nor do the cultures grown in liver-peptone broth in the modified Hall tube.

1. Holman and Krock: Am. J. Hyg., 1923, iii, 487.
2. Holman: J. Am. M. Ass., 1922, lxxviii, 1803.

## ADDENDA

### Group I

Organisms producing lesions resembling tuberculosis, chiefly in animals. Great variation in the morphology and cultural reactions of the different species. Usually non-motile.

The pseudotuberculosis group:

*Bacilus pseudotuberculosis rodentium* Pfeiffer, and its varieties.
" " *murium* Kutscher.
" " *ovis* (Preisz) Nocard.
" " *avium* (Muir).
" *orchiticus* (Kruse).
" *muris* Klein.
*Bacterium pseudotuberculare orchitophlogogenes* (Cagnetto).
DuCazal and Vaillard's human strain.
Wrede's bacillus.
Albrecht's bacillus.

#### Bacillus pseudotuberculosis rodentium Pfeiffer

Discovered by Pfeiffer[1] in a guinea-pig inoculated from a suspected case of glanders in horses and now recognized as the cause of wide-spread epidemics in rabbits and guinea-pigs. In these epidemics numerous nodules are found in the follicles of the intestinal mucosa, in the liver and spleen. These nodules have a firm capsule and fluid, creamy or purulent material inside. Microscopically epithelioid and lymphoid cells are found in this material, together with masses of bacteria which are easily stained in the early stages but later lose their capacity to take up dyes. The lesions resemble tubercles but may be differentiated by the absence of Langhans' giant-cells, and the lack of calcification. The organisms are possibly identical with those described by Malassez and Vignal[2] as the cause of "tuberculose zoogléique," with those found by Charrin and Roger[3] in pseudotuberculosis nodules in the liver and spleen of a spontaneously infected guinea-pig and by Nocard[4] in epidemics in poultry yards and in epidemics in rabbits. Closely related organisms are *Bacillus pseudotuberculosis*

*similis* isolated by Courmont[5] from the pleura of a tuberculous cow and named by Kruse, and *Bacillus opale agliaceus* of Vincenzi.[6]

**Morphology.**—Short plump coccus-like rods, about 1 to 2 $\mu$ long, often arranged in chains in broth cultures. Involution forms are common (Shigeshi and Kakehi[7]), small ring-shaped organisms with the centers less deeply stained than the periphery, filaments, rods with club-shaped bulges, etc. Rosenfeld[8] and Skschivau[9] report threads and branching forms on salt agar. No spores. Non-motile.

**Stained** with ordinary anilin dyes, best with alkaline methylene-blue or alkaline gentian-violet. The organisms often stain irregularly, especially in the tissues, showing bipolar granules. Gram-negative. Capsules have been reported by Shigeshi and Kakehi by Row-land's method for *Bacillus pestis*.[10]

**Cultivated** aërobically and anaërobically on the usual media at 22° C. and 37° C. The addition of glycerin and sugar favor the development of the organisms.

Fig. 125.—Pseudotuberculosis rodentium from plain agar three days old, stained with carbolfuchsin.

*Agar Plates.*—Good-sized colonies, somewhat like those of *Bacillus coli*, grayish-white, thick, and viscid. The plates have a disagreeable odor. Edges sometimes jagged (Shigeshi and Kakehi).

*Gelatin Plates.*—Deep colonies light colored with sharply defined edges, becoming darker in old cultures. Under low power they show central areas surrounded by concentric rings. Superficial colonies develop more rapidly, are grayish-white, at first sharply contoured and round, later provided with irregular edges. After a few days the center of the colony forms a papilla, pale yellow in color, surrounded by a wreath of crystalline material from the gelatin.

*Gelatin Stab.*—Grayish-white film along the line of inoculation. This gradually thickens, but leaves individual colonies on the edges. Later, a superficial thick disk-shaped expansion develops on the surface. No liquefaction.

*Coagulated Serum.*—Isolated watery drop-like pale opalescent colonies scattered over the surface. These colonies seldom coalesce, but tend to cease growth when they reach a certain size. More abundant growth on sheep serum than on beef or human serum.

*Potato.*—Yellowish-brown expansion, somewhat like that of the glanders bacillus.

*Alkaline Broth.*—No turbidity. Veil-like threads develop on the wall of the tube in forty-eight hours, gradually sinking to the bottom and leaving the broth clear. On shaking, a delicate film arises which also sinks to the bottom. Crystals similar to those formed in gelatin also develop in broth.

*Indol* not formed.

*Litmus Milk.*—Growth without visible change. Reaction alkaline according to Shigeshi and Kakehi.

*Carbohydrates.*—Poppe[11] finds that this organism does not ferment the sugars. Shigeshi and Kakehi, who worked over seven strains from the Lister Institute and two isolations from rabbits, report acid production from glucose, levulose, maltose, adonite, isodulcite, mannite, and glycerin, no acid from lactose, raffinose, saccharose, dulcite, sorbite, and inulin.

**Resistance.**—Destroyed in two hours at 60° C. and by drying in a desiccator forty-eight hours. Virulence lost in one hour at 60° C. The organisms survive some time at low temperatures.

**Pathogenic Action.**—Pathogenic to gray and white mice, guinea-pigs, rabbits, marmots, and hares. Horses, asses, goats, dogs, cats, hedge-hogs, rats, bats, field-mice, and birds are resistant. House mice die with progressive emaciation in fifteen to twenty days. At autopsy they show induration and pus formation at the site of inoculation, swelling and caseation of the lymphatic glands, metastatic granulations of cheesy consistency in the liver and spleen. Mice, guinea-pigs, and rabbits can also be infected by feeding. Guinea-pigs die after subcutaneous injection in five to six days, rabbits in twenty to twenty-five days. Male guinea-pigs injected intraperitoneally develop periorchitis (Straus's phenomenon). Intra-ocular injection in rabbits is followed by purulent panophthalmitis. The pathogenic action of this organism is not unlike that of *Bacillus mallei*, and Pfeiffer regards it as a pseudoglanders bacillus. No evidence of extracellular toxins.

1. Pfeiffer: Ueber die bacilläre Pseudotuberkulose der Nagetieren. Leipzig, 1889.
2. Malassez and Vignal: Arch. de physiol. norm. et. path., 1883, 3. s., ii, 369. *Ibid.*, 1884, 3. s., iv, 81.
3. Charrin and Roger: Compt. rend. Acad. de sc., 1888, cvi, 868.
4. Nocard: Compt. rend. Soc. de biol., 1889, 9. s., i, 608.
5. Courmont: Compt. rend. Soc. de biol. 1889, 9. s., i, 215.
6. Vincenzi: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1907, xliv, 391. *Ibid.*, 2. Abt., 1909, l, 2.
7. Shigeshi and Kakehi: J. Path. and Bacteriol., 1915–16, xx, 269.
8. Rosenfeld: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1901, xxx, 642.
9. Skschivau: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1900, xxviii, 289.
10. Rowland: J. Hgy., 1914, xiii, Plague Supplement, III, p. 418.
11. Poppe: Pseudotuberkulose. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, v, 775.

### Bacillus pseudotuberculosis murium Kutscher

Originally observed by Kutscher[1] in a spontaneous epidemic among mice. Similar epidemics were observed by Welch in the pathological laboratory of the Johns Hopkins Hospital. Careful studies of the organism found in one of these epidemics were made by Reed[2] who established its identity with Kutscher's organism. Present description taken chiefly from Reed's paper.

**Morphology.**—Single rods on young agar cultures, occasionally pairs and chains of three to four elements. Bacilli slightly thinner in cultures than in smears made directly from the animal body. Much variation in length, the average being below that of *Bacillus tuberculosis*. Small oval forms common in young cultures, which seem to elongate with age. Cultures twenty-four hours old show a few individuals with swollen and irregular features. In older cultures bizarre and dotted forms predominate. The rods tend often to form clump-like aggregations, the angular position and radiate (spoke-like) arrangement of which remind one of certain appearances noted in *Bacillus tuberculosis*. The bacilli often contain small round or oval refractive bodies indistinguishable morphologically from spores, but repeated heat tests showed that cultures containing them were no more resistant than those devoid of them. Branching forms observed from all films from agar cultures. Branching took place more abundantly at 40° C. than at 37° C. and was most pronounced on glycerin agar. The largest individuals were found on blood-serum. On coagulated egg-albumin (a poor medium) the bacilli remained small, stained irregularly and metachromatically, and branched frequently. Large involution forms and many branched individuals appeared on a medium containing butter. In branching, forked and Y-shaped individuals were frequently produced. The ends of the parent rod and the branch were often swollen, these thickenings presenting an unstained, sometimes quite refractive, center.

**Stains** well with methylene-blue, gentian-violet, carbolfuchsin, and Kutscher's carbol gentian-violet. No results with Neisser's differential stain. Gram-positive (Kutscher). Non-motile.

**Thermal Death-point** 60° C. five minutes. Organisms not capable of development after drying on silk threads ten weeks.

**Cultural Characters.**—*Agar Plates.*—Colonies small, light yellow in color. Under low power finely granular with sharply notched edges (Kutscher). Later the colonies lose their characteristic appearance, become round or whetstone in shape, and resemble colonies of *Streptococcus pyogenes.*

*Agar Slant.*—Grayish-white translucent growth in twenty-four hours, consisting of many minute discrete colonies more or less massed together. Growth more abundant at 37.5° C. than at 40° C. More abundant growth on glycerin agar and blood agar (Kutscher).

*Agar Stab.*—Line growth and surface expansion.

*Blood-serum.*—Growth like that on agar, but less abundant, scarcely perceptible at 40° C. No liquefaction.

*Gelatin Plates.*—Colonies like those on agar, but not yellowish. Dew-drop colonies on gelatin slopes, forming coherent growth only when thickly seeded.

*Gelatin stab* at 37.5° C. shows a slight growth without liquefaction. Frequent short lateral branches, described by Kutscher.

*Jensen's Medium* (broth with equal parts of human blood-serum).—Turbidity within fourteen hours, gradually clearing with the formation of a thready precipitate.

*Coagulated Egg-albumin.*—Dull growth at the end of twenty-four hours, at a temperature of 40° C.

*Plain bouillon* at 37.5° C., first cloudy and then clear, a precipitate forming in the bottom. Many crystals of ammoniomagnesium phosphate formed (Kutscher).

*Sugar-free bouillon* with neutral litmus showed evidence of growth, no change occurring in the color of the medium.

*Litmus bouillon* containing 1 per cent. glucose showed a rapid development of a decided pink color.

No acid in lactose-litmus-bouillon.

Acid formed in sugar-free neutral litmus bouillon, containing 10 per cent. perfectly neutral butter.

*Litmus Milk.*—Alkali production, a blue ring appearing at the surface.

*Potato.*—No growth.

**Pathogenic Properties.**—Slightly virulent to mice by subcutaneous injection. Intraperitoneal inoculation more successful, intrapleural inoculation uniformly so. Average duration of life thirty hours in intraperitoneal inoculation, fifty-four hours in intrapleural. At autopsy the pleura and pericardium are covered with a grayish exudate which exhibits a granular or finely lobular arrangement. Parietal pleura studded with minute translucent grayish nodules. These may coalesce into a continuous membrane which can be scraped or lifted off. The membrane is largely inflammatory. The nodules are often sharply circumscribed, and average from 0.5 to 3 mm. in diameter. The lungs may contain discrete nodules or a consolidated caseous mass. The diaphragm may also contain them, and the growth may extend and involve the peritoneum over the liver. Discrete nodules may also be found in the voluntary muscles, heart muscle, and in the subcutaneous tissues. The lymphatic glands and vessels may show tubercles or larger caseous masses. When bacilli gain entrance to the peritoneal cavity, minute tubercles cover all the serous surfaces, omentum, mesentery, and general serosa, while nodules are present in the spleen and kidney. No proof of tubercles in the liver. The joints may become affected, the synovial membranes being covered with tubercles and exudate. Microscopically the nodules are composed almost exclusively of bacteria with a few disintegrating cells or irregular masses of cells more or less fragmented. The tubercles are thus not tissue products, but merely colonies of bacteria. According to Reed there is grave question whether the pathological process should be called "pseudotuberculosis." The lesions produced experimentally by the inoculation of pure cultures into mice resemble closely the lesions found in the spontaneous cases from which the bacilli could be cultivated.

According to Kutscher this organism is pathogenic to gray and white mice. House mice can be infected by repeated inhalation, white mice are less sensitive, and field-mice resistant. No infection from feeding. Guinea-pigs, rabbits, dogs, cats, and birds are resistant.

## BIBLIOGRAPHY

1. Kutscher: Ztschr. f. Hyg., 1894, xviii, 327. *Ibid.*, 1896, xxi, 156.
2. Reed: Contributions to the Science of Medicine by the Pupils of William H. Welch. 1900, p. 525.

### BACILLUS PSEUDOTUBERCULOSIS OVIS (Preisz) Nocard

Originally reported by Preisz and Guinard[1] and described by Preisz[2] in the caseous foci in the kidney in a case of pseudotuberculosis of sheep. This is a common disease of sheep in France, in the Argentine, and in

Australia. It is sometimes called "caseous suppuration" by the French observers. The same organism was isolated by Nocard[3] from catarrhal inflammation of the lungs in calves and it is also regarded as the etiological agent of a disease among horses known as "ulcerative lymphangitis." Studies especially in America by Fischer and Hall[4] and Hall and Stone[5] in equine and bovine infections. In general the lesions are like those of pseudotuberculosis described in small animals and consist of small and good-sized nodules of caseous material inside a capsule or membrane of connective tissue. The organisms can be found in this material and cultivated from it.

**Morphology.**—Small bacilli in smears from the pus stained by Ziehl-Neelsen's carbol-fuchsin followed by 10 per cent. HCl or by Gram's method (Hall and Stone). The organisms are extremely pleomorphic, appearing as cocci, diplococci, diphtheroid bacilli, either in palisade arrangement or in irregular masses tending to form club-shaped or pear-shaped swellings. About 0.4 to 0.5 $\mu$ wide and 1 to 1.6 $\mu$ long on this medium. Swollen organisms, irregular in size and shape, are common. Non-motile. No spores.

**Stained** easily with the usual anilin dyes. Gram-positive. The diphtheroid forms on Loeffler's serum show metachromatic granules when stained by Loeffler's methylene-blue. Not acid-fast.

**Cultivated** on the usual media and isolated either directly from the pus or by the inoculation of guinea-pigs. Better growth under aërobic than under anaërobic conditions (Hall and Stone).

**Cultural Characters.**—*Agar Plates.*—Colonies appear after forty-eight hours at 37° C. as dry and disk-like with uneven edges and a folded or granular surface. After six to eight days these colonies reach their maximum and the largest are about 3 mm. in diameter, grayish-white in color. Development is better in ascites fluid agar. Glycerin hinders the growth if added in more than 2 per cent. concentration.

*Agar Stab.*—Striated growth along the line of inoculation made up of white isolated points.

*Blood Agar* (7.5 per cent. rabbit or horse blood).—Moderate growth. Colonies appear in twenty-four hours, circular, chalky, and opaque. On incubation for several days they are 8 to 10 mm. in diameter, and show a series of concentric rings about an elevated papilliform center. The growth is friable, the colonies breaking apart readily. Development is best at 37° C., but takes place at 20° C., although more slowly. In the absence of fermented carbohydrates the blood is hemolyzed and the agar clears. Lysis inhibited by fermentable sugars, a green pigment appearing in and about the colonies. This pigment is not sulphohemoglobin (Hall and Stone).

*Gelatin.*—Little growth at 22° C. In melted gelatin a moderate granular growth occurs at 37° C. The gelatin is not changed in consistency and solidifies in the ice-box.

*Coagulated Serum.*—Small colonies the size of poppy grains in forty hours at 37° C. These increase up to 1.5 mm. They have a smooth and shining surface, irregular borders, and a yellow, golden, or orange color. Great variation in the depth of the color. Abundant pigmented sediment in the water of condensation.

*Coagulated Egg-albumin.*—Growth like that on blood-serum, without the typical yellow color.

*Potato.*—No growth.

*Fluid Beef Serum.*—Abundant growth as thick flecks of yellow color producing a dense turbidity and a dirty-yellow pigmentation. After a time a thick yellow sediment forms on the bottom. The serum does not clear.

*Broth.*—No turbidity, granular sediment and dry friable surface pellicle like that of the diphtheria bacillus. No change in reaction. Little or no pellicle-formation in 1 per cent. glucose broth.

*Litmus Milk.*—No change.

Acid production in Hiss's serum water media containing glucose, levulose, and maltose (Hall and Stone). No gas formation. No acid in saccharose, lactose, raffinose, dextrin, inulin, glycerol, mannite, and dulcite.

Hemolysis on blood agar plates. The supernatant fluid from centrifugalized week-old broth cultures is slightly hemolytic for guinea-pig corpuscles. This hemolysin is not destroyed by boiling ten minutes.

**Resistance.**—Not destroyed by exposure to 55° C. for several hours. Destroyed in ten minutes at 65° C., in five minutes at 70° C. Survives on dried agar six months. Retains virulence for long periods in broth (six months, Carré[6]). Destroyed by $2\frac{1}{2}$ per cent. carbolic acid in one minute, by 0.25 per cent. formalin in six minutes, by 1 to 2 per cent. sublimate solution in four minutes. Not destroyed in twenty-four hours by lime-water (Grabert[7]).

When grown on agar containing gentian-violet according to Churchman's method,[8] the organisms are inhibited by a concentration of 1 : 10,000 (Hall and Stone). Dissolved in antiformin.

**Pathogenic** for white and gray mice, guinea-pigs, rabbits, goats, and sheep but not for fowls and pigeons (Poppe[9]). Mice succumb in two to four days after injection, with purulocaseous nodules in the peritoneum, liver, spleen, and kidneys. They succumb from feeding in two to four weeks. Guinea-pigs and rabbits die from subcutaneous or intraperitoneal inoculation in two to thirty-five days, depending upon the virulence of the culture. At autopsy a necrotic mass is found at site of inoculation and a generalized pseudotuberculosis with caseous nodules in peritoneum, lymph-glands, liver, and spleen. Male guinea-pigs inoculated intraperitoneally develop typical orchitis and periorchitis not unlike that seen in glanders. Guinea-pigs and rabbits succumb from intravenous inoculation in three to five days with generalized pseudotuberculosis. According to Panisset, guinea-pigs inoculated intracardially develop suppuration proceeding from this point with purulent osteoperiostitis and orchitis in male animals. Sheep develop only local tumefaction and swelling of the neighboring lymphatic glands from subcutaneous inoculation, but are easily infected by feeding or inhalation of pure cultures, and develop pseudotuberculosis. On this account it is usually believed that the disease usually arises spontaneously from taking in the organisms with food. In many cases chronic bronchopneumonia develops. The usual lesions found in infected animals are masses of "miliary pseudotubercles" which consist chiefly of round cells which early undergo necrosis. Older nodules show chiefly central caseation. These nodules occur chiefly in the liver, spleen, lungs, and lymph-glands. In sheep the infection tends to become chronic with pseudotubercles in the lungs and lymphatic glands and more rarely in the liver, kidneys, and muscles. In the larger lymph-glands the tubercles are encapsulated, caseous, containing greenish-yellow, viscid pus. The tubercles never show giant-cells. According to Dassonville,[10] Carré and Bigoteau[11] toxins are formed in cultures, 1 c.c. of the broth filtrate killing guinea-pigs in twenty-four hours. Rabbits are also sensitive, dogs and cats slightly, mice resistant. In sheep subcutaneous injection of small quantities is followed by local inflammatory edema while intravenous injection kills the animals in a few hours. The toxins are destroyed by heating. Carré and Bigoteau have immunized horses with this toxin, obtaining an antitoxin which protects guinea-pigs and sheep against fatal doses of the toxin but not against infection with the living bacilli.

1. Preisz and Guinard: J. de méd. vét. et de zootech., 1891, xlii, 563.
2. Preisz: Centralbl. f. Bakteriol. [etc.], 1891, 568 Ref. to Veternarius, 1891, No. 9.
3. Nocard: Bull. Soc. centr. de méd. vét., 1893, p. 116.
———: Ann. de l'Inst. Pasteur, 1896, x, 609.
4. Fischer and Hall: J. Am. Vet. M. Ass., 1915, xlviii, 18.
5. Hall and Stone: J. Infect. Dis., 1916, xviii, 195.
6. Carré: Revue gén. de méd. vét., 1910, xvi, 617.
7. Grabert: *In:* Handb. d. path. Mikrorog. (Kolle and Wassermann). 1903, iii, 751.
8. Churchman: J. Exper. Med., 1912, xvi, 211.
9. Poppe: Pseudotuberkulose. *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, v, 775.
10. Dassonville: Bull. soc. centr. de méd. vét., 1907, lxxxiv, 576.
11. Carré and Bigoteau: Revue gén. de méd. vét., 1910, xvi, 617.

### Bacillus pseudotuberculosis avium (Muir)

Isolated by Muir from pseudotuberculosis in birds. The liver and spleen contained numerous small yellowish nodules which microscopically appeared merely as finely granular masses without definite structure, made up of large numbers of short bacilli. Cultivated easily from the contents of the nodules.

**Morphology.**—Small bacilli 1.5 to 2 $\mu$ in length and about a quarter of this in thickness, short forms appearing like cocci and longer forms up to 5 $\mu$ in length. They appear singly, in pairs, sometimes in short chains, often in dense masses in which the individual organisms are distinguished with great difficulty. No spores. Non-motile. No flagella demonstrated.

**Stains** readily with the basic anilin dyes but loses the color very rapidly when decolorizing agents are employed. Quite decolorized by Gram's method. When stained somewhat faintly the extremities often appear deeply colored, the intervening portions remaining clear.

**Cultural Characters.**—Growth under both aërobic and anaërobic conditions, more rapid at 37° to 38° C. than at room temperature. Not killed at 60° C. in one hour, killed at 70° C. in the same time.

*Agar Slant.*—Whitish layer of growth, fairly thin and somewhat translucent, margins undulated. Single colonies have little tendency to become fused. Glycerin agar shows

the same type of growth as ordinary agar. When an agar culture is broken down in water, it tends to form little fragments suspended in the fluid, and does not readily give a uniform opalescence.

*Gelatin Plates.*—Superficial colonies thin, transparent films with irregular wavy margins, reaching 2 mm. in diameter on the third day, after which they do not undergo much enlargement. Under low power the colonies often show irregular darker points in the center, well-defined margins and slightly undulating and shining surfaces. Deep colonies spherical points of a slightly yellow color which, on the third day, reach scarcely 0.5 mm. in diameter. This size is never much exceeded. Under low power deep colonies have sharp circular margins of a brownish-yellow color and are slightly granular in appearance.

*Gelatin Stab.*—Growth visible in twenty-four hours as a whitish line which increases in thickness, the deeper part consisting of a row of spherical yellowish-white colonies. Usually on the third day at 20° C. a delicate surface layer is seen spreading out around the puncture. This increases in extent so as to form a round disk of transparent appearance which may reach the wall of the tube. The growth is closely similar to that of the typhoid bacillus. In all the cultures the deep part of the growth becomes brownish-yellow, and the superficial part more opaque. Occasionally feathery crystals are formed in the medium. *Stroke cultures* on *sloped gelatin* show a similar growth with somewhat sinuous margins. *Shake cultures* in *gelatin* give a general haziness in the medium without gas formation.

*Bouillon.*—Turbidity in twenty-four hours at 37° C., more marked below than above. Later the broth clears, a pretty thick layer of somewhat flocculent deposit gathers at the bottom, and a layer of growth adheres to the wall of the flask. Rarely flocculent masses gather on the surface. Broth cultures become alkaline.

*Milk.*—Growth but no visible change.

No acid or gas in *lactose gelatin.*

*Potato.*—Growth scanty or lacking, with heavy inoculations appearing as a somewhat transparent layer which becomes slightly yellowish in tint. It has slightly raised margins and does not spread far over the surface. It shows no resemblance to the glanders bacillus.

**Pathogenic Action.**—Virulent to small birds, canaries, linnets, etc. Subcutaneous inoculation of a drop or two of a suspension of a recent agar culture in water produces death in two and a half to six days. The lesions consist of swelling and inflammatory induration at the site of inoculation, a yellowish exudate under the skin, and extensive necrosis of the muscle extending from the surface downward. In addition there are nodules in the spleen and rarely in the liver. Feeding with portions of diseased organs or with agar cultures is also fatal, the animals dying in eight to thirteen days. At autopsy the spleen is enormously enlarged and studded with nodules of various sizes. The liver also shows a few scattered points. The postmortem appearance of animals killed by feeding is identical with the appearance in the natural disease. Virulent also to guinea-pigs, rabbits, and mice. In guinea-pigs intraperitoneal injection of $\frac{1}{2}$ to 1 c.c. produces death on the fourth to sixth day with a fibrinous exudate in the peritoneal cavity, small nodules the size of a pin-head in the mesentery and omentum, and numerous small yellowish nodules in the spleen and liver. With intravenous injection nodules appear in the spleen and liver, but apparently not in the kidney. Following subcutaneous injection there is a local inflammatory swelling which subsequently softens and caseates. A surface ulceration may occur, leaving an irregular ulcer with a caseous wall. The neighboring lymphatic glands are enlarged and nodules appear in the spleen and liver. Rabbits are susceptible, but less so than guinea-pigs, and the lesions are practically the same. White mice are also susceptible, but there is somewhat less tendency to the formation of nodules in the serous membranes after intraperitoneal injection.

**Remarks.**—Muir believes that his bacillus is probably the same as the bacillus of pseudotuberculosis of Nocard, Pfeiffer, etc. For the present it should be regarded as a separate organism.

Muir: J. Path. and Bacteriol., 1898, v, 160.

### BACILLUS ORCHITICUS (Kutscher) Kruse

Cultivated from the nostrils of a normal horse by Kutscher[1] and found to produce inflammation of the testicles in guinea-pigs. Apparently named by Kruse.[2]

**Morphology.**—Fine rods like the glanders bacillus showing unstained areas in stained specimens. Unstained material shows highly refractile irregularly divided bodies in the organisms. These stain intensely and are metachromatic granules. No spores. Non-motile.

**Stains** with difficulty by ordinary dyes. Gram-positive.

**Cultivated** easily on the usual media. All types of organisms destroyed at 55° C. in five minutes.

*Gelatin plates* slightly alkaline, at 22° C. show fine transparent colonies after twenty-four hours, visible only with a magnification of one hundred times. After forty-eight hours the gelatin looks stippled, due to the beginning liquefaction. Microscopically the colonies are small, colorless, finely granular, surrounded by an irregular zone made up of finer or heavier short processes penetrating the gelatin. On the third day the colonies are rounded off and have a yellow color. On thinly seeded plates the colonies have a dark yellow or brown color. Gelatin completely liquefied.

*Gelatin Stab.*—Delicate thread-like growth along the entire line of inoculation, with a narrow white surface colony which gradually sinks into the gelatin forming a typical air-bubble. The growth looks like a culture of the cholera vibrio.

*Plain or Glycerin Agar.*—Colonies in twenty-four to forty-eight hours round or whetstone in shape. When they break through to the surface they form an abundant white expansion. Microscopically the colonies are finely granular, yellow, with sharp edges.

*Agar Slant.*—Thick, white expansion in twenty-four hours.

*Agar Stab.*—Thread-like growth along entire line of inoculation with a thick, round colony at point of puncture which gradually spreads to the wall of the tube.

*Blood-serum.*—When first isolated the organisms developed a deep orange-yellow color. Later they lost their pigment production and produced white round colonies in twenty-four hours at 37° C. These slowly sank into the blood-serum and almost but never completely liquefied it. Older colonies caused a vapor-like turbidity of the surrounding medium.

*Potato.*—Thin, pure white, dry expansion in three to four days at 37° C.

In *peptone broth* and 1 per cent. *peptone salt solution* growth appears in twenty-four hours at 37° C. as small delicate floccules, which sink to the bottom without causing turbidity. Rarely a turbidity followed by clearing.

No growth in *milk*.

**Pathogenic Action.**—Intraperitoneal injection of male guinea-pigs with 2 to 3 oesa of a blood-serum culture produces a swelling of the testicle in forty-eight hours. The animals die on the fourth to fifth day. At autopsy the omentum is permeated with yellowish nodules and similar nodules are found in the testicles. Isolated nodules may also be present in the various abdominal organs. Sometimes those in the testicles are abundant and break down, giving rise to a purulent destruction of the substance of the gland. The pathological lesions in the testicle produced by this organism have the greatest similarity to the lesions produced by the glanders bacillus (Straus's reaction). Virulent also for gray mice and rabbits, not for pigeons.

1. Kutscher: Ztschr. f. Hyg., 1896, xxi, 156.
2. Kruse: *In:* Die Mikroorganismen (Flügge). 1896, ii, 455.

### Bacillus muris Klein

Described originally by Klein[1] as the chief organism found in 2 cases of pneumonia in white rats. Subsequently obtained by Mitchell[2] in pneumonitis and abscess formation in rats. In some instances the lungs showed areas of consolidation resembling lobar pneumonia. In the majority of cases abscesses were present varying in size from a pin-head to 2 cm. in diameter. They were scattered through the lungs and frequently occupied an entire lobe. Most of the abscesses were filled with a cheesy pus, although some were filled with a ropy mucus, a similar mucus being found in the bronchi. Cultures from the diseased lungs gave *Bacillus muris* almost constantly.

**Morphology.**—In fresh cultures short bacilli varying from 1.5 to 2.5 $\mu$ in length, most of them having an unstained band. Polar granules were present varying from three to five in number. Other organisms showed two or more unstained bands, and in some cultures, especially those under cultivation some time, granular forms predominated. The organism was slightly larger at one end, and the form having one unstained band resembled the pneumococcus very closely at first sight. Some solid forms were nearly always present. In very old cultures branching forms were found, simple lateral offshoots and various bizarre forms.

**Staining Reactions.**—The organisms stain well with the ordinary dyes, particularly Loeffler's methylene-blue. The granules were well-stained by Neisser's method.

Gram-positive.

**Cultural Characters.**—*Agar Slant.*—Growth along the line of inoculation as very small white granular colonies with a smooth border and little tendency to coalesce.

*Blood-serum.*—Granular colonies similar to those on agar, but larger. When widely separated the colonies become much larger.

*Gelatin Stab.*—Granular streak along line of inoculation. No liquefaction.

*Broth.*—Clouding of the medium in twenty-four hours. Upon standing the bacilli collect at the bottom of the tube. When the tube is shaken they diffuse readily through the medium.

*Hiss's Serum Water.*—Acid reaction in dextrose and maltose, but not in saccharose and lactose.

*Litmus Milk.*—No change.

**Pathogenic Action.**—Virulent to domestic rats, white rats, wild mice, and guinea-pigs. Subcutaneous injection is followed by abscess formation at the site of inoculation and metastatic abscesses in various regions, ventricles of the heart, diaphragmatic surface of liver, spleen, kidneys. Intraperitoneal inoculation produces purulent peritonitis with abscess formation and fistulas may form with a discharge of pus. Intrapleural injection is followed by purulent pleurisy with abscess formation, consolidation of various areas in the lungs, abscesses in the lung, and fibrinopurulent pericarditis. In the case of the guinea-pig the infection remains local. Occasionally septicemia develops in other animals. Microscopically, the abscesses consist chiefly of the organism in a kind of "Drusen" formation like that of actinomyces and the pseudotuberculosis bacilli.

Mitchell puts this organism in the group of diphtheroids or in the group of pseudotuberculosis bacilli. It differs from Kutscher's organism and Reed's organism by its staining by Neisser's method and Gram, by pathogenic action on the wild mouse, domestic rat, guinea-pig, and white rat.

1. Klein: Lancet, 1903, i, 238. Centralbl. f. Bakteriol. [etc.], 1903, xxxiii, Orig., 488.
2. Mitchell: J. Infect. Dis., 1912, x, 17.

### BACTERIUM PSEUDOTUBERCULARE ORCHITOPHLOGOGENES Cagnetto

Found by Cagnetto[1] in a spontaneous disease of guinea-pigs. The lesions in the abdomen resembled those described as "tuberculose zoogléique," and consisted of variously-sized small nodules on the surface and in the depths of the liver and spleen, in the mesentery, rarely in the thoracic organs. The nodules were white, soft, and full of milky fluid, consisting of mononuclear and polymorphonuclear leukocytes, together with bacilli in groups of twos and threes, chains, and filaments. When hardened and sectioned by Nicolle's method[2] it was found to have an outer membrane of fibrous tissue and a central mass of polymorphonuclear and embryonic cells, giant-cells like those found in tuberculosis and bacteria in groups of five or six. An organism was cultivated from these nodules which differed from those previously described.

**Morphology.**—Great variations in morphology. The organisms vary from coccoid bacilli to rods 3 $\mu$ long and 0.7 to 0.9 $\mu$ wide, straight or slightly curved, ends round, containing two to three vacuoles. They are often extremely irregular, comma-shaped, in chains of three to four like streptococci, or long filaments. Involution forms difficult to stain appear in agar five to six days old, earlier on agar containing blood. In broth fifteen to twenty days old long coarse bacilli occur almost like those of anthrax, with lateral embossments and terminal enlargements. Such organisms stain irregularly. On potato short, oval, or spherical bacteria like large cocci and staining deeply are found. Slightly motile. No spores.

**Stained** by ordinary dyes. Gram-negative.

Vitality and virulence maintained on agar five and a half months. No resistance to drying or to low temperatures. Dried on silk threads the majority of the organisms are dead in twelve days, all after sixteen days. Destroyed in forty-eight hours at 2° to 8° C., but not in fourteen hours.

**Cultural Characters.**—*Agar Plates.*—After thirty hours at 37° C. colonies like small translucent points to naked eye. Under the microscope these colonies show brownish granules in the center and a clearer sharp periphery made up of glistening scales disposed like fish scales. Colonies triangular or polygonal. Peripheral zones may be granular and pale. Contour always limited.

*Agar Slant.*—After thirty-two to thirty-six hours at 37° C. numerous small glistening, pearly colonies like pin-heads, which gradually coalesce and form a gray glistening undulating layer. Water of condensation very turbid.

*Agar Stab.*—After twenty-five to thirty hours slight growth along the stab as a delicate gray striation made up of punctiform or bristle-like colonies. On the surface a white glistening opaque mass surrounded by a diaphanous halo like the small petals of a daisy.

*Gelatin Stab.*—Abundant growth on the surface in two days, like the head of a pin, white and opaque, with a delicate thread along line of inoculation made up of small colonies.

Later filaments grow out at right angles and the superficial mass enlarges to a white disk with concentric circles. No gas. No liquefaction.

*Broth.*—Rapid growth with a uniform and intense turbidity, after thirty-six to forty-eight hours an iridescent pellicle on the surface. This later forms a white, thick, disk-like mass like a drop of wax which has been allowed to fall upon fresh water. On shaking this falls to the bottom without re-forming. In cultures six to eight days old an abundant whitish-gray sediment appears without floccules. In old cultures the fluid may be gray, opaque, or orange. No clearing. No odor.

*Glycerin Broth.*—Less rapid growth, slight turbidity, with a few white granular floccules adherent to walls of the tube or deposited on the bottom. Acidity in glucose broth. No conversion of saccharose to dextrose. No indol.

Little or no anaërobic growth in broth, in an atmosphere of hydrogen. Only after five to six days a few very small white floccules along the walls of the tube made up of masses of bacteria.

*Milk.*—Abundant growth without change in reaction, but with a gradual alteration of the constituents. After six to eight days at 35° C. the upper part of the milk becomes transparent, has the color of milk-serum, while the lower part is white, flocculent, and made up of coagulated casein. On shaking the sediment is partially dissolved without a return of the natural color to milk. The fluid preserves a dirty-gray or yellowish color.

*Potato.*—After forty-eight hours a moist expansion, gray and diaphanous. Later this becomes pale yellow and orange-yellow. It resembles a culture of the glanders bacillus on potato, but does not turn chocolate-brown. The growth remains flat and dry, has a metallic lustre, and is limited to the line of inoculation. No growth on potato to which acid, alkali, or glycerin is added. Abundant growth on veal serum, horse serum, and on agar mixed with human blood. No pigment formation.

**Pathogenic Properties.**—Virulent to guinea-pigs, pigeons, and white mice. Subcutaneous inoculation in guinea-pigs of small quantities is followed by large local nodules and a nodular formation in all the organs. The animals die usually in six to eight days. Intraperitoneal inoculation produces signs of infection in two days and an orchitis with a more or less intense vaginalitis. The disease usually begins in one testicle and extends into the other. The skin of the scrotum becomes tense, glistening, and slightly reddened. The testicle enlarges to the size of a small nut and becomes adherent to the surrounding tissues. The death of the animal occurs between the fifth and sixth day and at autopsy there is a diffuse fibrinous purulent peritonitis with an enormous swelling of the spleen and a bilateral vaginalitis, the two layers of the tunica vaginalis being joined by a layer of fibrinous exudate varying in thickness. Large quantities of culture introduced intraperitoneally kill guinea-pigs rapidly without producing orchitis. Infection can also be produced by feeding, the animals dying in forty to sixty days and showing at autopsy purulent glands in the mesentery, a nodular infiltration, sometimes ulcerating, in the follicles of the intestine. Pigeons die rapidly after intravenous inoculation of small quantities, ten to fifteen days after subcutaneous inoculation, the lesions resembling those in guinea-pigs injected in the same way, that is, numerous gray tuberculiform nodules in all the internal organs and large, very compact masses at the site of inoculation. In white mice the lesions vary from small abscesses at the site of subcutaneous inoculation which ulcerate and slowly cicatrize, or small abscesses which lead to general infection with the formation of small specific nodules in the liver, spleen, and lymphatic glands of the abdomen. The animals die in twenty to twenty-five days. The organism has little effect on rabbits and cats, producing only an edematous tumefaction at the site of inoculation, and no effect on dogs. The nodules produced experimentally have the same histological structure as those in the natural disease.

1. Cagnetto: Ann. de l'Inst. Pasteur, 1905, xix, 449.
2. Nicolle: Ann. de l'Inst. Pasteur, 1892, vi, 783.

### Bacillus of DuCazal and Vaillard

Isolated by DuCazal and Vaillard from a human case showing caseous nodules in the peritoneum and pancreas, one in the liver, and an adherent subacute peritonitis. Microscopically these lesions showed amorphous caseous material without cellular elements and small bacilli which differed from the organism producing pseudotuberculosis in animals.

**Morphology.**—Short bacilli in the tissues, with round ends, slightly longer than broad, isolated or joined together. Somewhat smaller in cultures, isolated, in pairs or in chains of ten to fifteen or more elements. No spores.

**Motility** oscillating and vibratory.

**Stains** uniformly with Loeffler's blue, aqueous gentian-violet and aqueous fuchsin, or

shows distinct polar staining. True capsules may be demonstrated by these dyes. Gram-negative.

**Cultivated** as a facultative anaërobe at 37° C. Slow growth at 15° C.

**Cultural Characters.**—*Agar.*—Moist, opalescent, translucent growth in twenty-four hours at 37° C., extending rapidly from the line of inoculation and covering the whole surface by a delicate uniform layer. Eventually the growth becomes slightly thicker, but remains transparent.

*Gelatin.*—Punctiform whitish colonies along line of inoculation in twenty-four hours. Abundant growth on surface. Slow liquefaction from above downward. On top of the non-liquefied gelatin a white flocculent deposit.

*Broth.*—Turbidity in twenty-four hours, whitish sediment, thick, and flocculent. The liquid has a milky consistence and is very alkaline.

*Potato.*—Abundant, thick, projecting, humid, slightly viscous growth, at first clear yellow then yellow-brown. Surface rapidly covered.

Odor from all cultures slightly ammoniacal, like putrid urine.

Viable in open cultures several months. In closed cultures viable a year.

Destroyed at 60° C. in fifteen minutes. When dried resists 100° C. five minutes.

**Virulent** to white mice and rabbits. No action on guinea-pigs. In white mice $\frac{1}{8}$ c.c. broth culture several days old produces death in forty-eight to sixty hours from septicemia. In rabbits, intravenous injection of 1 c.c. fresh culture produces death in thirty-six to fifty hours from fetid diarrhea and paresis, and septicemia. Small doses, $\frac{1}{2}$ c.c. injected intravenously, produce a chronic disease, diarrhea and transitory emaciation, followed by tumors in various parts of body like the caseous nodules in the original disease. They are usually subcutaneous and were found out once in the thoracic cavity. This infection was always fatal.

DuCazal and Vaillard: Ann. de l'Inst. Pasteur, 1891, v, 353.

### Wrede's Bacillus

A number of cases of disease in man have been described as pseudotuberculosis but the first definite case of this character is that of Aschoff,[1] thoroughly studied and reported by Wrede.[2] In a two-year-old child Wrede found at autopsy numerous tubercle-like nodules in liver, adrenals, lungs, pharynx, esophagus, and intestine in which acid-fast organisms could not be demonstrated. From the liver a non-motile, Gram-positive short rod was isolated, which showed a tendency to produce involution forms. This produced a uniform turbidity in broth and no formation of crystals, thus being differentiated from *Bacillus tuberculosis rodentium.* Agar and gelatin cultures were likewise free from crystals and had no disagreeable odor.

*Pathogenic* for white mice, guinea-pigs, and rabbits by subcutaneous, intraperitoneal, and intravenous inoculation. No infection from feeding. At autopsy nodules are found in liver and adrenals but not in spleen, kidneys, or lungs. These nodules show necrotic masses and the bacilli but no giant-cells.

Wrede believes that a true pseudotuberculosis exists in man caused by non-acid-fast organisms.

1. Aschoff: Festschr. d. 73. Versamml. d. deutsch. Naturf. u. Aerzte. Hamburg, 1901.
2. Wrede: Ziegler's Beiträge, 1902, xxxii, 526.

### Albrecht's Bacillus

Isolated by Albrecht from a suppurating area in the abdominal wall removed at operation. Belongs to the group of pseudotuberculosis bacilli. Pathogenic for mice, guinea-pigs, white rats, and rabbits by inoculation, for guinea-pigs by feeding. At autopsy the animals show lesions like pseudotuberculosis or plague. Infection believed by Albrecht to have come from cats.

Albrecht: Wien. klin. Wchnschr., 1910, xxiii, 991.

## Group II

Miscellaneous pathogenic or obligatory saprophytic bacteria not already described:

*Bacillus maximus buccalis* Miller.
*Bacterium binucleatum* Swellengrebel.
*Bacillus vaginæ* Kruse.
" of *soft chancre* Ducrey.
Zur Nedden's *bacillus*.
*Diplobacillus* of Morax-Axenfeld.
" *liquefaciens* (Petit).
*Bacillus duplex non-liquefaciens* Scarlet.
" *duplex Josephi* of Scarlet.
" *pseudo-œdematis maligni* Sanfelici.
" *aureus fœtidus* Herzog.
" of *equine acne contagiosa* Deeckerhoff and Grawitz.
" *pyelonephritidis bovis* Enderlen and Höflich.
" *polymorphus suis* Degen.
" *nephritidis equi* Meyer.
*Bacterium nephritidis* var *ovis* Fitch and Beaver.
*Bacillus ranicida* Ernst.
" *hydrophilus fuscus* Sanarelli.
" *septicemiæ ranarum* Venulet and Padlewski.
*Coccobacillus fœtidus ozenæ* Perez.

### Bacillus maximus buccalis Miller

Described originally by Miller[1] from the material collecting on the outer side of the teeth, more rarely on the inner side. Miller reported it as a large rod with rounded ends, 1.4 to 1.9 $\mu$ wide and of great length, varying from 8 to 27 $\mu$. It frequently appears in long chains. Not cultivated. Extensively investigated by Swellengrebel,[2] who used moist emulsions in gelatin, or fixed emulsions dried on coverslips. The best results were obtained with Schaudinn's sublimate alcohol fixative, or 35 per cent. formalin, the preparations being stained with Heidenhain's iron hematoxylin, Romanowsky, or aqueous methylene-blue (1 : 10).

The cell-walls stain sharply in methylene-blue. Division by transverse fission, two septa extending in from the outer wall and dividing the cell into two daughter cells. These septa really are a ring like that observed by Bütschli, Meyer, and Migula. Division takes place in the central or peripheral part of the organism. In the living organism granules and vacuoles may be seen. Stained with dilute iodin solution containing potassium iodid violet, amyloid-like bodies appear which are probably iogen. The vacuoles may really be filled with iogen. In organisms stained by Heidenhain's iron hematoxylin many vacuoles appear in single or double rows. If the preparations be sufficiently decolorized, peripheral and central granules may be made out connected by fine threads running obliquely to the long axis of the cell. These threads really form a spiral running through the cell and are composed of chromatin and plastin. There are three to five spirals in each cell, but the number of granules is inconstant. The granules stain blue in methylene-blue and red by Romanowsky-Ziemann. They also are chromatin. They stain red with a mixture of methylene-blue and 50 per cent. sodium bicarbonate as true nuclei do, and dark blue with methylene-blue, 1 to 10, followed by iodin potassium iodid, and brown with iodin. Swellengrebel believes that the spiral band with its granules is a structure analogous to the nuclei of higher plants and animals. It seems to divide in cell division, each half going to a daughter cell.

1. Miller: Die Mikroorganismen der Mundhöhle. Leipzig, 1892.
2. Swellengrebel: Centralbl. f. Bakteriol. [etc.], 1906, 2. Abt., xvi, 617; 673.

### Bacterium binucleatum Swellengrebel

Originally observed by Miller in the mouth and named by Swellengrebel. A short, plump, curved rod, somewhat thinned and rounded at one end. After transverse division it has a length of about 1.2 $\mu$ and grows to a length of about 5.1 $\mu$ before it divides. Breadth 0.6 to 1.2 $\mu$., non-motile. Plasmolyses with difficulty.

Plasma has no internal structure, no vacuoles, and stains light blue or reddish-blue by Giemsa. Cell wall stains light red by Giemsa. The protoplasm contains two large bodies which stain darker than the surrounding protoplasm and appear light red by Giemsa. These bodies are spherical or disk-shaped and have a diameter which is usually about that of the cell. They may lie close to each other or at either end. These are regarded by Swellengrebel as true nuclei. They divide with the division of the parent cell and each daughter cell has again two nuclei. They are neither volutin, fat, or amyloid-like substance by chemical tests. They give most of the reactions of chromatin. Finally during division these chromatin masses undergo changes characteristic of nuclear division.

Swellengrebel: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1907, xix, 193.

### Bacillus vaginæ Kruse

Described by Doederlein[1] as the vaginal bacillus. Named by Kruse.[2] Obtained by Doederlein from the vaginal secretions of normal pregnant women and of virgins, and regarded as the cause of the acid reaction.

**Morphology.**—Medium-sized, fairly slender, non-motile, non-flagellated rods in smears from vaginal secretions. Gram-positive. In cultures often in long threads which show Gram-negative areas, together with involution forms (Rother[3]).

**Cultivated** with considerable difficulty. Original cultures obtained by Doederlein by planting secretions rich in organisms in 1 per cent. sugar broth, incubating twenty-four hours at 37° C. Lactic acid produced in sugar media. Krönig[4] was able to obtain cultures only in *glucose agar* incubated anaërobically. Cultivated recently by Rother who found that it grew under both aërobic and anaërobic conditions, but only at or near the body temperature. Best growth on *sugar media* and in *liver broth*, which naturally contains considerable amounts of carbohydrates, and in *liver agar*. Colonies on agar show thready prolongations which are apt to fail in old cultures. The addition of serum or ascites fluid favors growth only if glycogen be present in the media. Growth on both acid and alkaline media. Media should not be diluted. Acid formation marked. In sugar media this runs to an amount equivalent to 150 c.c. normal acid to the liter. This is usfficient to kill staphylococci, streptococci, and *Bacillus coli* in twelve to twenty-four hours.

Dried on silk threads viable after twelve months. Killed at 60° C. in twenty minutes and at 70° C. in six minutes.

According to Rother *Bacillus vaginæ* does not attack glycogen directly, but only after it is inverted. Possibly in the menstrual period the glycogen may be changed to sugar by the blood ferments and appear in the vaginal mucosa.

1. Doederlein: Das Scheidensekret und seine Bedeutung für das Puerpiralfieber. 1892.
2. Kruse: *In:* Die Mikroorganismen (Flügge). 2. Aufl. 1896, ii, 358.
3. Rother: Centralbl. f. Bakteriol. [etc.], 1. Abt. 1922, lxxxviii, 558.
4. Krönig: Centralbl. f. Gynäk., 1895, xix, 409.

### Ducrey's bacillus of soft chancre

Obtained by Ducrey[1] by the inoculation of the arm with material from a soft chancre. Arm-to-arm inoculation was carried to the fifteenth generation. After five or six generations the pus contained but one kind of organism. Ducrey's observations were confirmed by Krafting,[2] who found the same organism in the pus from a large number of ulcers two to three days after inoculation. Also called the "streptobacillus" of Ducrey.

**Morphology.**—Non-motile bacilli, about 0.4 $\mu$ in thickness and 1.5 $\mu$ in length (Park, Williams, and Krumwiede[3]). In the pus they have a characteristic parallel arrangement, lying side by side, either inside the pus-cells in groups of two to six or outside in shorter or longer chains which may be agglutinated in tufts like plaits of hair. The organisms show great

diversity of form. According to Fischer,[4] when stained by Unna's polychrome methylene-blue and differentiated with a mixture of glycerin and ether, the organisms appear as short, curved, homogeneous rods with rounded ends measuring 0.4 $\mu$ in breath by 1.5 to 1.7 $\mu$ in length, dumb-bell forms, diplococcus forms 0.3 to 0.4 $\mu$ in breadth by 1 to 1.5 $\mu$ in length, short rods almost like cocci 0.3 to 0.35 $\mu$ broad and 0.4 $\mu$ long, and bacilli with central unstained oval areas measuring 0.5 to 0.6 $\mu$ in breadth by 1.1 to 1.5 $\mu$ in length.

*Stains* best with carbolfuchsin. Often shows polar granules. Gram-negative. Stained best in tissues by polychrome methylene-blue or by the Unna-Pappenheim method with fixation in alcohol or formalin and staining with methyl-green.

**Cultivated** with some difficulty. Cultures obtained first by Lenglet[5] on agar containing two parts of peptone made from digested human skin, the surface of the agar being covered with human blood. Subsequently cultivated by Istamanoff and Aspianz[6] on agar made from a filtrate of pulverized and macerated human skin. Grows best on agar containing one to two parts of human, rabbit, or dog's blood (Besançon, Griffon, and LaSourd,[7] Park, Williams, and Krumwiede). The organisms grow first in the water of condensation, usually in association with other bacteria. Pure cultures may be obtained later on the surface of blood agar plates. On the second or third day small isolated colonies appear about 1 mm. in diameter. Later they increase somewhat, are about 2 mm. in diameter, raised, somewhat spherical or disk-shaped. They are colorless by direct light, grayish-white by transmitted light, intensely viscid. No change in the color of the medium, no hemolysis or methemoglobin production. Growth also in uncoagulated blood-serum (rabbit) with a slight clouding of the medium and small flakes, and on coagulated serum.

Cultivated most successfully by Teague and Deibert[8] in Zinsser's laboratory in small quantities (1 c.c.) of rabbit blood, allowed to clot at room temperature, and heated to 55° C. for five minutes. The organisms grow in characteristic form in the serum. Pure cultures can be obtained by transfer to blood agar plates with a pH of 7.2 to 7.3. Teague and Deibert obtained positive results in 140 out of 274 persons.

**Resistance** slight. The organisms may survive several weeks on blood agar at 37° C., but soon die in cultures on coagulated serum. Especially sensitive to deleterious influences, disinfectant, etc.

**Pathogenic Action.**—Not virulent to laboratory animals. Characteristic soft chancres produced in man by inoculation of pure cultures by Fischer, Lipschutz,[9] and Tomasczewski.[10] With the secretions of the ulcers Nicolle[11] has produced corneal ulcers and keratitis in rabbits by the inoculation of the cornea.

1. Ducrey: Monatsh. f. prakt. Dermat., 1889, ix, 387.
2. Krafting: Arch. f. Dermat. u. Syph., 1892, ii, 41.
3. Park, Williams, and Krumwiede: Pathogenic Microörganisms. 1920, p. 468.
4. Fischer: Inaug. Dissert. Berlin, 1903.
5. Lenglet: Bull. Soc. de Dermat., 1898, ii, Ann. de dermat. et syph., 1901.
6. Istamanoff and Aspianz: Protokol der Kaiserl. Kaukas. med. Ges., 1897, 1, xii, No. 10.
7. Besançon, Griffon, and LaSourd: Ann. de dermat. et syph., 1901.
8. Teague and Deibert: J. Urol., 1920, iv, 543.
9. Lipschutz: Arch. f. Dermat. u. Syph., 1905, lxxvi, 77.
10. Tomasczewski: Arch. f. Dermat. u. Syph., 1880, xii.
11. Nicolle: Recherches sur le chancre mou. Thèse de Paris, 1893.

### Zur Nedden's bacillus

Found in isolated instances in corneal ulcers, ulcus serpens, and keratitis by Zur Nedden,[1] McNab,[2] and others.

**Morphology.**—Straight or slightly curved rods often arranged as diplobacilli, 0.9 $\mu$ in length, 0.6 $\mu$ in thickness. Threads rare. Ends rounded. With weak staining central or peripheral light areas appear, possibly vacuoles. No chains. Gram-negative. No capsules. Non-motile.

**Cultivated** easily in ordinary laboratory media. Optimum temperature 37° C. Sparse growth at 10° C. and at 40° C. Destroyed at 55° C. in three and four-tenths hours. An obligate aërobe. It grows on *agar* after twenty-four hours as slightly raised, round, circumscribed colonies, light bluish by transmitted light. These coalesce to a thick viscid layer. In plates the superficial colonies are large, round, with small darker central areas showing concentric markings by transmitted light. Deep colonies are small, round, or whetstone-shaped. No growth in the depths of *agar stabs*.

*Gelatin Plates.*—Transparent bluish homogeneous colonies.

*Gelatin Stab.*—Growth only in upper part with the formation of a flat nail-head mass.

*Sugar Agar.*—Acid production. No gas.

*Milk* coagulated.

*Broth.*—Slight growth. No indol.

*Potato.*—Thick yellow-brown expansion.
*Loeffler's Serum.*—Thick gray-white growth.
*Human Blood-serum.*—Thick gray-white expansion.
Very slightly resistant to drying. Destroyed at 55° C. Produces keratitis experimentally in rabbits.

1. Zur Nedden: Arch. f. Ophth., 1904, lxix, 360.
2. McNab: Ulceration of the Cornea, 1907.

## Diplobacillus of Morax-Axenfeld

First observed in subacute conjunctivitis by Morax,[1] and within a few months found independently by Axenfeld[2] and cultivated on Loeffler's blood-serum. Since then the organism has been found in chronic conjunctivitis in many different parts of the world.

**Morphology.**—Present in the viscid secretion of the conjunctiva in great numbers, sometimes free, sometimes in the cells, and sometimes on the outside of the epithelial cells. Found but rarely in leukocytes since this type of conjunctivitis is relatively free from pus-cells. Usually in pairs, short chains, and long chains, often made up of pairs of organisms. The individuals are usually 2 to 3 $\mu$ long, 1 to 1.5 $\mu$ broad. They may appear as diplococci or as large cocci. In cultures on Loeffler's serum; during the first two days the organisms appear as diplo- or streptobacilli of different sizes, not unlike those in the secretions. In older cultures large involution forms occur, diplobacilli, chains, threads of varying length, and club-shaped organisms.

**Staining Reactions.**—Stained by ordinary anilin dyes. Gram-negative. Polar staining appears sometimes in organisms in the secretions. In old cultures the protoplasm is irregular, slightly granular. These granules stain dark blue with Neisser's stain (Marongu[3]). Capsules have been described by various authors (Gifford[4]). Axenfeld states that the organism has a narrow ectoplasm which occasionally looks like a true capsule. The majority have no capsules.

**Cultivation.**—Cultivated only at 37° C. on *blood-serum*, *serum agar*, or media containing body fluids. On *Loeffler's serum* (made from beef or sheep) after twenty-four hours small, moist, sunken, transparent places appear which gradually deepen till the whole medium is slowly liquefied. The color of the medium may remain the same or become brownish. If the turbid fluid mass is removed, the serum looks as if eaten or nibbled. When the colonies are separated they form larger deep holes in the medium, when the colonies lie closely together they coalesce, the thick viscid material running down and collecting in the water of condensation. After fourteen days the entire medium may be liquefied. On *serum agar* the colonies are small, transparent, flat drops of delicate grayish color like pneumococcus colonies. Under low power they are finely granular, round with smooth edges, slightly prominent, do not coalesce, and when single are not so large as colonies on Loeffler's serum. In *serum-broth* a delicate diffuse turbidity with a fine sediment appears which on shaking is easily dislodged. The broth may become slightly acid and the organisms die.

The organisms grow best on alkaline media, somewhat on neutral, little or not at all on acid media. They do not grow in the presence of staphylococci which make the media acid, but will grow with the xerosis bacillus which renders the medium alkaline. They grow well in the presence of the ordinary white staphylococci of the conjunctiva. They do not grow on potato, sugar agar, gelatin, or in milk (McNab).

They die out rapidly in cultures and seldom survive more than eight days. Destroyed at 55° C. in five minutes. Dried on sterile splinters of wood they remain infectious for four days. In the secretions not destroyed by sixty hours' exposure to bright light (Colomb). Dried on sterile linen they can be cultivated after twenty-four hours (Erdmann).

**Pathogenic Action.**—Non-pathogenic to animals. Placed on the human conjunctiva pure cultures produce characteristic conjunctivitis (Axenfeld, Morax, Hoffmann[5]). Rymowitsch[6] reports that injection into the anterior chamber of the eye in animals is followed by a plastic iritis. If glass beads are also introduced an abscess forms.

1. Morax: Ann. de l'inst. Pasteur, 1896, x, 337.
———: Ann. d'ocul., Janvier, 1897.
2. Axenfeld: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1897, xxi, 340; 343.
———: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, vi, 587.
3. Marongu: Recherche batteriologiche nelle congiuntiviti e dactiocistite. Clin. ocul., 1910, ii, 188.
4. Gifford: Ann. ophth., 1898.
———: Ophth. Rec., 1905, xiv, 511.
5. Hoffmann: Arch. f. Ophth., 1899, xlviii, 638.
6. Rymowitsch: Post. okul., 1900, No. 9.

### DIPLOBACILLUS LIQUEFACIENS Petit

Described originally by Petit[1] in conjunctivitis with corneal ulcers.

**Morphology.**—In the secretions, isolated bacilli, or diplobacilli with rounded ends, slightly smaller than the diplobacillus of subacute conjunctivitis of Morax-Axenfeld, 1 to 1.5 $\mu$ in width by 2 $\mu$ in length. Bacillary forms also resembling Friedländer's bacillus. No capsules. In broth cultures twenty-four hours old the organisms appear more like diplococci. In cultures four to five days old, large involution forms are present staining unevenly. Non-motile. Capsules reported in cultures by McNab.[2]

**Stains** uniformly by ordinary anilin dyes. Gram-negative.

**Grows** readily on all laboratory media.

Optimum temperature 20° to 37° C.

*Ascites Agar Slant* (2 parts agar, 1 part ascites fluid).—Grayish, thick, viscid growth. Under hand-lens regular, round, projecting.

*Ascites Agar Stab.*—Surface growth only, in form of a thick whitish colony.

*Plain Agar.*—Same type of growth except a little more abundant.

*Coagulated Serum.*—Abundant liquefaction in three to four days.

*Gelatin Slant.*—Liquefaction along the line of growth, 2 mm. wide in twenty-four hours, 4 to 5 mm. in forty-eight hours.

*Gelatin stab* at 15° C. Slight cup-shaped surface liquefaction, reaching the walls of the tube in six to seven days.

*Gelatin Plates.*—Deep colonies rare. Surface colonies, 1.5 to 2 $\mu$ wide in twenty-four hours, round, whitish-yellow. Under hand-lens such colonies are seen to be made up of an opaque center surrounded by a more transparent radiating zone. Under low power of the microscope they are granular, brownish-yellow and opaque in the center, which shows several small patches. The center is surrounded by a more transparent radiating zone, more whitish-yellow, with irregular edges, arborescent, disappearing into the medium.

*Bouillon* (peptone 2 per cent.).—Only slight turbidity in lower part, with heavy inoculation.

*Ascites Bouillon.*—Abundant turbid growth in twenty-four hours at 35° C. Turbidity increases, a deposit forms at the bottom, and an opaque pellicle on the surface. Numerous particles adhere to the sides of the tube.

*Milk.*—No growth and no coagulation.

*Potato.*—Slight whitish-yellow expansion, creamy and viscous.

Aërobic development only in ascites agar, sugar agar, and sugar ascites agar.

Vitality of eight to ten days in gelatin at 12° to 15° C.

Destroyed at 50° C. in twenty minutes, at 55° C. in fifteen minutes.

Non-pathogenic for ordinary laboratory animals.

Inoculated into the cornea of a rabbit it produces a local reaction, characterized by a grayish infiltration and an injection of the iris which disappears rapidly.

1. Petit: Recherches clinique et bactériologique sur les infections aiguës de la cornée. Thèse de Paris, 1900, p. 227.
2. McNab: Monatsbl. für Augenheilk., Jahrg. 42, 1904, i, 54.

### BACILLUS DUPLEX NON-LIQUEFACIENS Scarlet

Isolated by Scarlet[1] from conjunctivitis with corneal ulcer. Found by Oliver and Wherry[2] in mucopurulent sputum in acute and chronic bronchitis where it was often as abundant as the pneumococcus.

Gram-negative bacillus, arranged as a diplobacillus. Stains well by Pick and Jacobson. Non-motile.

Grows as an aërobe and also under partial tension.

**Cultural Characters.**—*Ascites Agar Slant.*—Numerous colonies in twenty-four hours at 37° C., small, round, half transparent, about 1½ mm. in diameter. Periphery transparent, center opaque and elevated.

*Gelatin Stab.*—After forty-eight hours delicate half transparent grayish-white growth along line of inoculation. No liquefaction. Gradual increase in size, reaching surface in four days. At end of six days surface growth is 1½ mm. in diameter, line growth also increased in thickness. By the eleventh day surface colony 2¼ mm. in diameter, by fourteenth day 5 mm. By the thirtieth day it reaches the walls of the tube, covering entire surface. No liquefaction.

*Coagulated Serum Slant.*—Round, stippled, half transparent colonies in twenty-four hours. No erosion or liquefaction. On next day colonies thicker, slightly more opaque. Much the same after a month. No liquefaction or erosion.

*Plain Agar Slant.*—Half transparent colonies in twenty-four hours with opaque centers and clear peripheries. Thicker in forty-eight hours. At room temperature colonies appear in four days.

*Broth.*—Uniform turbidity in twenty-four hours, increased in forty-eight hours.

No manifest growth in *milk*. No coagulation.

No growth on *potato* or *carrots*.

On *litmus agar* containing dextrose or maltose, colonies are round, half transparent, about ½ mm. in diameter. No change in color. No fermentation. Agar colonies in Petri plates in twenty-four hours are round or oval, grayish-white, finely granular, opaque center, transparent periphery ¼ to ½ mm. in diameter.

*Ascites Agar.*—Luxuriant growth at 37° C. under partial tension with the hay bacillus. Diplobacillus arrangement retained (Oliver and Wherry).

*Killed* in five minutes at 50° C.

Injection of organism into anterior chamber of eye of rabbits produces only a local reaction which heals rapidly. No ulcers produced. Not virulent to mice or rabbits.

Immune serum in rabbits agglutinates *B. duplex non-liquefaciens*, but not the diplobacillus of Morax or the diplobacillus of Petit.

1. Scarlet: Ann. d'ocul., 1916, cliii; 100, 485.
2. Oliver and Wherry: J. Infect. Dis., 1921, xxviii, 341.

### Bacillus duplex Josephi of Scarlet

Obtained by Scarlet from conjunctivitis with corneal ulcers.

Diplobacilli like Morax diplobacillus of subacute conjunctivitis. Non-motile.

Gram-positive.

*Growth* on *ascites agar* in twenty-four hours as raised grayish knobs, half opaque, 2 to 2.5 mm. in size.

*Gelatin.*—No growth. No liquefaction.

*Coagulated Serum.*—Abundant growth. No liquefaction.

*Bouillon.*—Marked turbidity, delicate, non-coherent film.

*Ascites Bouillon.*—Growth like that in ordinary bouillon.

*Plain Agar.*—Colonies spread out and thick.

No growth in *milk* or on *potato*.

Scarlet: Ann. d'ocul., 1915, cliii, 100.

### Bacillus pseudo-œdematis maligni Sanfelici

This organism was apparently first isolated by Klein[1] and subsequently obtained by Sanfelici[2] who gave an accurate description of it and proposed to call it *Bacillus pseudo-œdematis maligni*. Similar organisms were found by Chavigny[3] and probably the same species was obtained from 2 cases of gas gangrene in Welch's laboratory and studied by Lanier.[4]

Sometimes called the aërobic variety of *Bacterium Welchii*.

According to Klein the organism has the following characters:

**Morphology.**—Bacilli with round ends isolated or in short chains. The single organisms measure about 0.7 μ wide by 0.8 to 2.4 μ long.

Actively motile.

No spores.

Gram-negative.

Organisms killed at 70° C.

**Cultural Characters.**—*Surface colonies* in twenty-four hours at 20° C. are small, gray, round points. In forty-eight hours larger, flattened, with thin, irregular edges. After a few days they are several millimeters wide.

*Deep colonies* round, regular, brownish, the size of a pin-head.

*Agar Slant.*—Moist, gray-white, abundant growth.

*Gelatin Stab.*—Whitish growth along line of inoculation with punctiform colonies about the lower part. Smooth, grayish, transparent expansion at the point of puncture with irregular margins.

*Broth.*—Turbidity and flocculent sediment. No scum.

*Potato.*—Light yellow, viscid growth.

*Glucose Gelatin Shake Culture.*—Abundant gas production, gas bubbles appearing about each colony in the depths.

*Glucose Gelatin Stab.*—Abundant development along line of inoculation, gas bubbles in the depths.

**Pathogenic Action.**—Pathogenic for mice, guinea-pigs, and rabbits. Subcutaneous inoculation of $\frac{1}{4}$ to $\frac{1}{2}$ c.c. kills guinea-pigs in twenty-four hours with an extensive subcutaneous edema. The organisms isolated in Welch's laboratory had the power of producing gas abundantly in the blood and tissues of rabbits which were inoculated intravenously and then killed in a few minutes. It also caused the same spreading emphysematous necrosis in pigeons and guinea-pigs as is produced by *Bacterium Welchii*.

1. Klein: Centralbl. f. Bakteriol. [etc.], 1891, x, 186.
2. Sanfelici: Ztschr. f. Hyg., 1893, xiv, 352.
3. Chavigny: Ann. de l'Inst. Pasteur, 1897, xi, 860.
4. Welch: Johns Hopkins Hosp. Bull., 1900, xi, 185.

### BACILLUS AUREUS FŒTIDUS Herzog

Isolated by Herzog from the liver and heart's blood in a suspected case of plague.

**Morphology.**—Short bacilli with rounded ends, varying much in size. They are from 0.6 $\mu$ to 2 $\mu$ long; on an average the bacilli vary from 0.55 $\mu$ to 0.8 $\mu$. Proportion of length to thickness usually 2 to 1. The organism presents itself frequently as a diplobacillus. A large number of them are short, making them look much like diplococci. Occasionally there are found small individuals which are not materially larger than 0.5 $\mu$ and almost spherical, so that it is hard to distingiush them from true diplococci. The bacilli possess a capsule of moderate size, which can be demonstrated by Muir's method. They do not form large chains, even groups of four in a chain being but rarely seen. Spore-formation is not observed. The organism is not motile.

**Staining Properties.**—The bacilli are readily stained by the watery anilin stains and easily overstained by the more intense solutions (carbolfuchsin, carbol-thionin). When lightly stained, not all of the bacilli take the stain uniformly; but in some cases the stain acts in such a manner that an unstained space remains in the center. It is not demonstrable that this polar staining is due to the presence of Ernst-Babes polar granules, because Neisser's methylene-blue Bismarck brown stain does not show any such granules satisfactorily, though there appears to be a slight tendency at the poles of the bacillus to take up some of the blue stain. The bacillus resembles somewhat the short type of the pseudodiphtheria bacillus. A certain similarity also exists between this bacillus and that of plague, though the similarity is not great. Gram's method decolorizes the bacillus.

**Cultural Peculiarities.**—The organism produces on all solid media tried an intense golden-yellow pigment, which is practically identical in color with the pigment formed by *Staphylococcus pyogenes aureus*. Distinct colonies in 20 per cent. *gelatin plates* are quite difficult to obtain, because the organism liquefies the gelatin with great rapidity. Twenty per cent. *gelatin stab* cultures after twenty-four hours are largely fluidified. The liquefaction comprises the entire extent of the upper strata. There is some growth along the line of the stab, but not much. The liquefied gelatin looks very cloudy and after twenty-four hours a dense scum is formed on its surface. Individual colonies are best studied on *agar plates*. On *agar* and *glycerin agar* the organism after twenty-four hours forms a moist, raised, golden-yellow growth. The individual colonies are more or less round and likewise moist and raised, with a somewhat undulating surface. The margins are smooth. In spreading, the colonies become confluent. The development on *glucose agar* is identical with that on ordinary agar. No gas formation occurs. On 3 per cent. *salt agar* the growth is possibly a little slower, although not very much so. The bacilli raised on this medium stain as usual, and do not show the involution forms so characteristic of the plague bacillus. On *lactose agar* the growth is similar to that on the other agars. There is no gas formation. The development on the surface is more rapid than in the depth of the stab. In *lactose litmus agar* the color begins to turn after twenty-four hours and is quite distinctly red after forty-eight hours. *Broth* after twenty-four hours is strongly clouded, and after forty-eight hours a pellicle has been formed on the surface. On *potatoes* typical luxuriant growth is observed after twenty-four hours. *Litmus milk* is slightly reddened after twenty-four hours, and is strongly so after forty-eight hours. Coagulation takes place only at the end of several days. The organisms develop typically under anaërobic conditions in both a nitrogen and a hydrogen atmosphere. All cultures whether aërobic or anaërobic have a fetid, cheesy, and somewhat cadaverous smell.

The thermal death-point of the organism was determined to be 62° C. An exposure of ten minutes at this temperature destroyed all the bacilli, while 61° C. acting for ten minutes left a number alive.

Non-pathogenic to the gray rat, rabbit, and monkey (*Macacus philippinensis*).

Herzog: J. Infect. Dis., 1905, ii, 49.

### Bacillus of equine acne contagiosa Dieckerhoff and Grawitz

Cultivated by Grawitz and Takishige from cases of a peculiar infectious pustular disease of the skin in horses. About half as long as the tubercle bacillus and only 0.2 $\mu$ broad. Forms long oval and round spherical bodies which are well-stained by Gram's method. The rods are straight or slightly bent, often in twos, end-to-end, or parallel.

**Stains** by fuchsin, less well with gentian-violet or methyl-violet, but not with methylene-blue.

*Grows* best on blood-serum at 37° C. where many punctiform white colonies develop in twenty-four hours. White colonies on gelatin plates, in gelatin stab a thread of spheres. No growth on potato. No growth under 17° C. Neutral or slightly alkaline reaction most favorable. Abundant growth of small colonies on egg-albumin. No fermentation of glucose or lactose.

Destroyed at 80° to 90° C. Survives a long time in the dried scales.

*Virulent* to horses, sheep, calves, dogs, and small laboratory animals. Subcutaneous injection may produce a phlegmon in dogs, mice develop pyemia after subcutaneous inoculation, but do not react to the organism when it is merely placed on the skin. Guinea-pigs are exquisitely sensitive, developing a hemorrhagic erysipelatous lesion of the skin from local application of a culture and dying in forty-eight hours. In horses lesions are produced which resemble those found in the natural disease, swelling of the skin with pea-sized pustules.

Dieckerhoff and Grawitz: Virchow's Arch., 1885, cii, 148.

### Bacillus pyelonephritidis bovis Enderlen and Höflich

Observed first in pyelonephritis of cattle by Damman and Rivolta and carefully described by Enderlen, and by Höflich[2] in 1891. The organisms are not specific for the disease which may be due to a variety of organisms. Same as *Bacillus renalis bovis*.

**Morphology.**—Small non-motile bacilli, 2 to 3 $\mu$ long by 0.7 $\mu$ broad, sometimes slightly curved, ends round or thickened, appearing almost exclusively in bunches. No spores. Stained best by Gram's and Weigert's methods.

Grows only under aërobic conditions, best at 37° C., but little at room temperature.

**Cultivation.**—*Agar Plates.*—Small punctiform colonies with sharp edges.

*Agar Slant.*—At 37° C. in twenty-four hours small grayish points forming a narrow zone along line of inoculation, with edges somewhat raised. The colonies are adherent to the agar. Under low power the young colonies are round brownish-gray points with sharp edges, centers and peripheries showing no differentiation in color or appearance. The colonies seem to consist of fine brownish-black, thickly intertwined rods. Older colonies have irregular peripheries and under high magnification individual twisted short threads are visible penetrating the agar.

*Agar stab* shows a fine streak, grayish-white in color, with delicate spheres along the edges. Slight granular growth at point of puncture.

*Blood-serum.*—At 37° C. in twenty-four hours, fine gray punctiform colonies which are somewhat larger than those on agar. The growth is about 1 mm. wide. The granular colonies are close together and only slightly raised from the surface of the serum. Water of condensation clear with a fine sediment which clouds the fluid on shaking.

*Milk.*—No growth.

*Broth.*—Fine sediment, supernatant fluid clear. On shaking, this sediment renders the broth turbid.

*Potato.*—No growth.

No growth on *gelatin*.

Growth frequently glistening and viscid.

**Pathogenic properties** uncertain. Purulent inflammation occasionally in mice and guinea-pigs on subcutaneous inoculation. According to Kitt inoculation into the anterior chamber of the eye is followed by iritis which heals.

1. Enderlen: Deutsche Ztschr. f. Thiermed., 1891, 325.
2. Höflich: Monatschr. f. prakt. Tierh., 1891, 337.

### Bacillus polymorphus suis Degen

Isolated by Degen from purulent nephritis in swine.

**Morphology.**—Short rods and threads. Non-motile. Gram-negative.

Grows aërobically best on *slightly alkaline agar* containing peptone. Colonies delicate, gray, slow-growing, reaching their maximum in three to four days and remaining small. Viscid precipitate in water of condensation. Colonies adherent to the surface of agar. The organisms are transferred with great difficulty and die out after six days. Slight development in *gelatin stab* without liquefaction. No growth on *blood-serum*. In *broth* a uniform turbidity which clears leaving a precipitate which on shaking rises in a cue-like mass.

No change in *milk*. *Litmus whey* first reddened with subsequent alkaline reaction. On *potato*, dirty-gray, sharply circumscribed, glistening colonies. Acid, no gas in glucose, saccharose, and lactose.

Hydrogen sulphid positive. Indol negative.

Slightly pathogenic for mice, producing only a local abscess. Large intraperitoneal doses produce serofibrinous peritonitis in guinea-pigs, fatal in twenty-four hours. Rabbits show symptoms of severe illness, but recover.

Degen: Untersuchungen über die hæmatogene eitrige Nephritis des Schweines. Inaug. Dissert. Geissen, 1907.

### BACILLUS NEPHRITIDIS EQUI Meyer

Found in the kidneys in purulent nephritis of horses by Meyer.[1] Described as *Bacterium viscosum equi* by Magnusson[2] from joint-ill of horses.

**Morphology.**—Rather long thick bacillus with rounded edges in the pus, staining with difficulty. Length 0.5 to 0.8 $\mu$. Rarely the organisms showed bipolar staining and filaments. In twenty-four-hour glycerin agar cultures, the bacilli were in parallel bundles and showed sharply cut ends. In six-day-old cultures, short and cocci-shaped forms were frequent. After repeated cultivation the organisms appeared as long filaments, probably an involution form. In milk and bouillon cultures, fine, rather long bacilli were more common. In cultures the pleomorphism of the bacillus was more marked than in tissues. No motility. No flagella. No spores.

**Stains** best with dilute fuchsin, gentian-violet, or Giemsa solution. Films treated with dilute acetic acid showed a swollen, irregular mucous capsule. Gram-negative. In pus stained best with Loeffler's methylene-blue. No metachromatic bodies.

**Cultural Characters.**—*Gelatin Plates.*—Slow growth, small punctiform grayish shiny soft colonies appearing in two to three days, strongly adherent to the surface of the medium. Maximum size a pin-head. Ten days later colonies slightly sunk in the medium and surrounded by a small quantity of diluted and liquefied gelatin. Colonies themselves more convex and dull. Under a magnification of 50 diameters the colonies were round, finely granular, with sharp or slightly irregular margins. In colonies ten to twelve days old the edges showed fine filaments protruding into the surrounding liquefied zone.

*Gelatin Stab.*—Nail-like growth, filiform along the needle-track, showing, after five to six days, fine, punctiform, white, discrete colonies and regular lateral branches. Colonies did not become confluent. When ten to twelve days old the "head" had a diameter of 2 to 5 mm. was irregular, lobulated, with folded margins, and slightly yellowish in color. From the seventh day a slight liquefaction commenced in the upper part of the needle-track and a small bell-shaped depression formed with its maximum size on the fourteenth day. The deposit of the liquid mass was whitish and flocculent and the whole "nail" seemed to sink into the gelatin.

No growth on *plain agar plates*.

*Glycerin Agar Plates Slightly Alkaline.*—Small, whitish, shiny, slightly conical colonies about the size of a pin-head in twenty-four hours, very adherent to the medium. Direct isolation from the body showed only a few very large and very shining colonies. After forty-eight hours colonies were only slightly increased in size; they had a white center and slightly yellowish edges. Both large and small colonies were very viscid, of mucous consistence. Long filaments of mucus were easily drawn off by the platinum loop 30 cm. in length. No deep colonies. Under a magnification of 50 diameters the colonies appeared round with a regular margin, convex, dark, and opaque. The margin adhered to the medium. The larger colonies had a transparent periphery which was slightly granular while the center was homogeneous and gray.

On *glycerin agar slants* colonies developed which showed a marked tendency to expand, but were not confluent. They were very mucoid and adhered to the medium. Water of condensation was mucous and very stringy, forming a whitish mass which after twenty-four hours separated into a clear upper part and a whitish deposit. The upper part was viscid and did not moisten the glass. Growth reached maximum in forty-eight hours.

*Glycerin Agar Stab.*—Filamentous, whitish, slightly granular streaks of growth appeared along the needle-track in twenty-four hours, surface irregular and very slimy.

*Serum Glycerin Agar.*—Growth more rapid and abundant when horse serum used. Colonies very soft and larger than on other media.

*Urine Agar.*—Growth very slow, colonies showing a distinct brown discoloration.

*Brain Agar.*—Growth slow like that on glycerin agar.

*Glycerin Agar Shake Cultures.*—Few round white colonies on the surface or only 1 cm. below.

*Ascites Agar* or *Pleural Exudate Agar.*—Good growth like that on serum agar.

*Liquid Serum* (horse, cattle, sheep, dog).—Thick compact deposit in eighteen hours, the whole medium viscid and mucous.

*Coagulated Serum.*—Fine, clear, round colonies in twenty hours, rather adherent, not confluent. Water of condensation very viscid with a whitish flocculent deposit. Formation of mucus more distinct when grape-sugar or glycerin was added. Strings up to a length of 1 meter could be withdrawn.

*Glycerin Bouillon.*—Slight turbidity and distinct deposit in twelve hours. In twenty-four hours the upper part was clear and the deposit was rather tenacious and of mucous consistence.

*Glycerin Peptone Solution.*—Growth poor. Better growth and a more viscid deposit when serum is added.

*Grape-sugar Bouillon.*—Growth like that in glycerin bouillon. No gas. Reaction acid. Abundant mucous formation in bouillon to which 1 per cent. glucose, mannose, galactose, and fructose were added. Only slight mucous formation with maltose, saccharose, lactose, erythrite, dulcite, and mannite.

*Blood Agar* (horse or rabbit blood).—Small, slightly reddish, slimy colonies. No hemolysis. Colonies four days old showed a dirty-greenish discoloration.

*Potato.*—No growth.

*Milk.*—After thirty-six hours milk became viscid, adherent, and mucous. Typical coagulation sixty-four to seventy hours afterward. On the fifth day a flocculent adherent deposit of casein coagula appeared in the bottom of the tube and the upper part of the medium was very viscid.

*Petruschky's Litmus Whey.*—Acid reaction typical red in color in forty-eight hours, with a viscid mass on the bottom.

*Lactose Litmus Agar Plates* (Conradi-Drigalski).—Viscid adherent colonies in twenty-four hours, surrounded by a red zone. After forty-eight to sixty-four hours plates almost entirely acid.

*Endo-fuchsin Agar.*—Deep red colonies in twenty-four hours, older colonies slightly fluorescent.

No growth under 20° C., poor growth at 22° C.

Optimum temperature 37° C.

Obligate aërobe.

No growth in media without albuminoids.

No indol. No $H_2S$. No odor. No gas.

Acid from glucose, mannose, galactose, fructose, lactose, raffinose, maltose, dulcite, and mannite.

Destroyed quickly at temperatures over 50° C. and by diffuse daylight in twenty to thirty-five minutes. Not especially resistant to chemical disinfectants, sublimate and lysol solutions killing it at once.

**Pathogenic Action.**—Found in the purulent lesions in the kidneys, lungs and in the urine in the natural disease in horses. Pure cultures produce abscesses in horses and mules by subcutaneous injection, the kidneys showing yellowish nodules at autopsy in killed animals. Intravenous inoculation produces death in three to six days from pyemia and purulent nephritis. Direct injections into the anterior chambers of the eye produces purulent panophthalmia. Liquid cultures kill animals in a very short time, twelve to twenty-four hours, probably by toxemia. Intraperitoneal injection of guinea-pigs and rabbits produces fibrino-purulent peritonitis, but not regularly. Not virulent to mice, rats, dogs, sheep, cattle, or birds. Complement fixation test given by the serum of infected animals.

This organism shows similar morphological and cultural characters to *Bacillus polymorphus suis* found by Degen[3] in swine and to *Bacillus polymorphus* of Grixomi.[4]

1. Meyer, K. F.: Transvaal Department of Agriculture, Report of Government Veterinary Bacteriologist, 1908–09, p. 122.
2. Magnusson: J. Comp. Path. and Therap., 1919, xxxii, 143.
3. Degen: Untersuchungen ueber die hæmatogene eitrige Nephritis des Schweines. Inaug. Diss., Giessen, 1904.
4. Grixomi: Riforma Med., Nos. 235–237, pages 111, 122, 134.
———: Baumgarten's Jahresbericht, 1896, xii, 512.

### BACTERIUM NEPHRITIDIS VAR. OVIS Fitch and Beaver

First isolated from ovine nephritis by Fitch and Beaver. Somewhat resembles *Bacillus nephritidis equi* of Meyer.

**Morphology.**—Short rod with rounded ends, 1 to 2 $\mu$ in length. Occasionally coccoid forms are found especially in old slant agar cultures, and long involution forms are noted on agar. Non-motile.

*Stains* readily and uniformly with anilin dyes. Gram-negative.

**Cultural Characters.**—Growth very scant on *beef infusion agar*, especially when first isolated. Growth more vigorous on agar containing 10 per cent. horse serum. Colonies discrete, grayish-white, translucent and show a bluish iridescence. After four generations of artificial cultivation the growth becomes more confluent and filiform. Strains differ somewhat in heaviness of growth. No growth on *gelatin* at 20° C. At 37° C. growth slight without liquefaction. Only a few discrete colonies appear on the surface. In *meat-infusion broth*, especially in early generations, growth is sparse, becoming visible in forty-eight hours. In later generations growth is moderate in twenty-four hours. Cloudiness is uniform with a semiviscid white sediment which adheres to the sides of the tube and forms a viscid terminal ring. In broth containing certain carbohydrates a thin surface pellicle is formed. In five-day cultures the sediment is quite heavy. Sparse growth in Dunham's solution. No indol. Small white colonies on Loeffler's *serum*. No liquefaction. No change in *plain* or *litmus milk*. No hemolysis on defibrinated *blood agar plates*.

**Fermentation Reactions.**—Strains differ in their ability to act on carbohydrates. One strain isolated by Fitch and Beaver produces acid in the hexoses, dextrose, levulose, galactose, in maltose and in mannitol, arabinose, and zylose. No action on rhamnose, sucrose, lactose, raffinose, salicin, or dulcitol. No gas. The other strain produces no acid in carbohydrates with the possible exception of xylose.

**Pathogenic Action.**—No action on rabbits and guinea-pigs from subcutaneous inoculation. Intravenous inoculation of the material from two twenty-four-hour serum cultures of the acid-producing strain was fatal at times, the kidneys showing parenchymatous degeneration. The non-acid producing strain has only a slight action on animals. The two strains do not appear to be related immunologically.

Fitch and Beaver: J. Infect. Dis., 1921, xxviii, 345.

### Bacillus ranicida Ernst

Described by Ernst from the blood in an epidemic disease of frogs in Heidelberg.

**Morphology.**—Slender bacillus about the size of the typhoid bacillus in the blood and organs, about 1 to $2\frac{1}{2}$ $\mu$ long rarely 3 $\mu$ long. More slender than the typhoid bacillus, not so fine and delicate as the organism of mouse septicemia. On agar about the same size, on potato long rods 7 to 8 $\mu$, but no long threads. No spores. In old cultures on agar the organisms contain very refractile, glistening, oval bodies.

**Stains** well in aqueous methylene-blue or Löeffler's methylene-blue. Gram-negative.

**Cultivated** easily on the usual media.

*Agar* at 22° C. growth appears as a slightly raised flat streak several millimeters wide in twenty-four hours. In forty-eight hours the growth consists of a homogeneous viscid expansion which has a greenish color, not fluorescent. At 30° C. the growth is more abundant in twenty-four hours and has jagged edges. In forty-eight hours the growth is thicker, a film developing on the water of condensation. Little or no growth at low temperatures (8° C.). No growth at the body temperature (38° C.). Optimum temperature 20° to 30° C.

*Gelatin.*—Rapid growth and liquefaction along the entire line of inoculation. The zone of liquefaction is several centimeters wide on the fourth to fifth day and has a greenish color, not fluorescent. On the surface is a thin film which is easily broken and sinks to the bottom forming a turbid cloudy precipitate. In old cultures the color becomes somewhat brownish.

*Potato.*—Light brown, raised expansion, the potato in the neighborhood assuming a dirty-green color.

**Pathogenic Action.**—Virulent to *Rana esculenta*, less so to *Rana tempororia*. Inoculation of pure cultures into the lymph sac brings about the death of the animals in about two to five days, the organisms being found in the blood and organs. The animals apparently are more susceptible in spring than in summer, and when kept artificially at low temperatures than at high temperatures.

Ernst: Beitr. z. path. Anat. u. z. allg. Path., 1890, viii, 203.

### Bacillus hydrophilus fuscus Sanarelli

Originally cultivated by Sanarelli[1] from water and found to be pathogenic for frogs and for warm-blooded animals. Isolated in this country by Russell[2] from "red leg" of frogs and later by Emerson and Norris[3] who made a complete study of its properties and its pathogenic action.

**Morphology.**—A fairly thick rod from the blood of infected frogs, showing marked bipolar and irregular staining by Jenner's blood stain. In fresh blood smears it commonly appears as a shovel-shaped bacillus with square ends strikingly fringed, giving the picture of a diplobacillus. In young cultures on all media shorter and longer forms predominate while coccoid forms and diplobacilli are also present. The organism is thicker than the typhoid bacillus, straight, the length usually five to six times the breadth. In older agar cultures (four days to several weeks), and on potato, the rods remain short and thick, although filaments and poorly staining forms are occasionally met with in abundance. After several days to a week's growth on coagulated ox serum the organism becomes markedly filamentous and long rods and bizarre involution forms are frequent. No spores.

Organisms from young cultures stain intensely with all the anilin dyes. Gram-negative.

Young cultures very actively motile. Single wavy terminal flagella about six times the length of the bacillus demonstrated by Russell in his strain.

**Cultural Reactions.**—Growth rapid and abundant on the usual media, optimum temperature being about 37° C. Growth also abundant at room temperature and at temperatures as low as 12° C.

*Agar colonies* on the surface are whitish, somewhat raised and moist, coarsely stippled. Other portions of the colonies are pale and have even borders, central portions decidedly yellowish and marked by round aggregations. Deep colonies round or oblong, deep yellow in color, irregular, lobulated.

*Agar Slant.*—Moist whitish streak spreading rapidly, the growth assuming a yellowish tint in the course of time. Pigmentation more marked in the early than in the later subcultures. The yellowish pigmentation of the early cultures becomes intense after several weeks.

*Gelatin Plates* (12 per cent. gelatin, 2 per cent. acid to phenolphthalein).—Colonies yellowish by transmitted light, irregularly marked. When small and young, they are pale and slightly stippled. Well separated large colonies cause advanced liquefaction within two days.

*Gelatin Stab.*—A small, globular-shaped area of liquefaction develops beneath the surface of a gelatin stab culture. The liquefaction increases slowly in extent and is not complete for several weeks. A whitish pellicle is soon formed and a heavy, whitish deposit settles out promptly. No greenish tint. Gelatin stabs grown for two days and then melted remain fluid at room temperature. After three days' growth the gelatin remains fluid when placed in cold storage. Filtrates of both young and old broth cultures peptonize gelatin rapidly and completely.

*Milk.*—Completely clotted in twenty-four hours at 37° C. Liquefaction of the clot is apparent in four days and is practically complete in ten days. At room temperature clotting and liquefaction occur more slowly than at incubator temperature.

In *litmus milk* (1.5 per cent. acid to phenolphthalein) a marked acid reaction promptly develops. The coagulum may be finely divided and then settles out promptly leaving the fluid clear. The clot never completely disappears and may or may not retain its acid color. In milk rendered alkaline (0.2 c.c., n/1 NaOH to 10 c.c.) the reaction becomes acid and a finely divided clot appears in two days at 37° C. This is soon liquefied and the fluid becomes clear. In milk rendered acid (0.2 c.c. n/1 HCl to 10 c.c.) the same succession of changes is observed. Sterile filtrates of broth cultures bring about changes in milk similar to those caused by the living bacillus.

*Broth.*—Turbidity in twenty-four hours, and flocculi and a heavy pellicle soon develop.

*Potato.*—(Natural reaction, 0.5 per cent. acid to phenolphthalein.) At room and incubator temperature growth is abundant, wet, slightly raised and yellowish. This yellow color changes in a few days to a reddish hue, assuming later a rusty-colored sputum tint. In four weeks the potato is covered by a heavy and dull brownish-colored growth, the upper and dry portion of the growth presenting a greenish metallic lustre. On potatoes rendered alkaline by 0.2 per cent. NaOH the growth never turns reddish in color, but is abundant, the early citron color turning slowly into a brownish-gray. On potatoes acidified by HCl growth is scanty and invisible.

*Coagulated Ox Serum.*—Abundant whitish growth. No pigment formation. Gradual softening of the serum, the liquefaction in the tubes kept at 37° C. being concealed by the evaporation.

**Fermentations.**—When freshly isolated the organism obtained by Emerson and Norris fermented dextrose (1 per cent.), mannite, and saccharose with the production of acid and some gas, a large bubble developing in the course of several days at 37° C. Both arms of the fermentation tubes showed abundant growth. After several generations on agar the bacillus lost its power of fermenting sugars with gas production. The property of growing in the closed arm of the fermentation tube containing sugar-free broth was retained, but in a lesser degree, a turbidity developing after twenty-four hours at 37° C. Abundant growth in the open arm of the tubes containing sugar-free broth and lactose broth, a heavy whitish deposit settling out in a few days. Growth in the open arm of the tubes containing dextrose,

mannite, and saccharose not nearly so abundant as in the lactose and sugar-free broth. Cloudiness in both arms similar.

In Hiss's serum media, 1 per cent. dextrose-, mannite-, saccharose-, maltose-, dextrin-, and starch-litmus serum water (1 to 3) become firmly clotted in twenty-four hours at 37° C. The clot in the dextrose tubes remains solid, in the other sugars the clot is softened after three days; in dextrin it is completely liquefied. Clot is an "acid" clot. In 1 per cent. lactose and in 1 per cent. inulin-litmus serum water (1 to 3) the color, as in plain sugar-free litmus serum water, remains unchanged, no acid reaction being developed. The serum water becomes opaque and finally solid in four days. Softening takes place slowly, the clot being almost liquefied in three weeks, while the litmus is decolorized. This clot is a "sweet" clot due to a rennet-like ferment.

No splitting of *urea* or *asparagin* with acid production. Nitrates reduced to nitrites and indol slowly produced in Dunham's peptone.

**Pathogenic Action.**—Virulent to frogs (*Rana esculenta, tigrina, viridis*, etc.) in small doses ($\frac{1}{8}\frac{1}{0}$ of a twenty-four-hour agar culture). The animals show edema, dulness, red leg, and superficial ulceration. They may die acutely (forty-eight hours) and at autopsy show skin lesions, hemorrhagic papules and occasional vesicles, with large hemorrhagic exudates. The dorsal and ventral lymph sacs are distended with colorless or blood-tinged fluid. Hemorrhages are also present on the tongue, in and between the muscles, in the periosteum. The muscle tissue is softened. The stomach is distended with viscid ropy mucus and markedly congested. Heart sometimes pale but usually healthy. Spleen and liver soft and mottled. Lungs show no lesions. Blood shows advanced degenerative changes in the red cells. The animals may also develop a more chronic intoxication, dying in fifteen days. They show less edema, but the same visceral lesions and marked blood changes. In *rabbits* agglutinins and precipitins develop after inoculation with emulsions of living bacilli or filtrates of broth cultures. The organisms isolated by Emerson and Norris were but slightly virulent for rabbits. The original strain obtained by Sanarelli was virulent for rabbits as well as the strain obtained by Russell.

Sanarelli's strain was virulent for toads (*Bufo cinereus*), salamanders (*Triton cristatus*), lizards (*Lacerta agilis* and *viridis*), fishes (*Barbus plebejus*), fresh water eels (*Anguilla vulgaris*), guinea-pigs, rabbits, puppies, kittens, white mice, field-mice, pigeons, and chickens. Russell's strain was virulent for frogs, guinea-pigs, rabbits, house mice, and white rats. Two toxic substances were obtained by Russell, resembling digitalis and veratrin in their action, one insoluble in alcohol and one soluble in alcohol.

1. Sanarelli: Centralbl. f. Bakteriol. [etc.], 1891, ix, 193.
2. Russell: J. Am. M. Ass., 1898, xxx, 1442.
3. Emerson and Norris: J. Exper. M., 1905, vii, 32.

### BACILLUS SEPTICEMIÆ RANARUM Vénulet and Padlewski

Isolated by Vénulet and Padlewski from an epidemic disease of frogs.

Short and longer rods with rounded ends like *Bacillus typhosus*. In films from infected material usually in pairs, no long threads, tending to show bipolar staining like the plague bacillus. Stains by ordinary dyes. Usually Gram-negative.

Motility due to single terminal flagella. Capsules in films from the organs of frogs. No spores.

**Cultivated** easily on the usual media.

*Agar Slant.*—Transparent expansion like that of the members of the typhoid group, with a pale bluish shimmer by reflected light.

*Agar Colonies.*—Transparent, glistening, colorless, with smooth edges 2 to 3 mm. in diameter. Colonies do not become turbid.

*Bouillon.*—Turbidity, cloudy on shaking. No film. Small sediment after three to four days.

*Gelatin.*—Liquefied above in one to two days like a culture of the cholera vibrio. After three to four days a small layer of liquefied gelatin.

*Clotted Blood-serum.*—Liquefied.

*Milk.*—Clotted after three to four days.

*Bouillon* of Omeliansky colored with neutral red (Berestneff's method) shows some gas formation and late loss of color.

*Grape-sugar agar* shows gas bubbles.

*Peptone water* with mannite and litmus somewhat red in two days with a reddish sediment.

The same reaction with grape-sugar. No change in color with lactose.

Indol positive.

**Pathogenic** to frogs, which die in two to three days. At autopsy the lymph sinuses and abdominal cavity are filled with turbid opalescent hemorrhagic fluid, the muscles are edem-

atous and hemorrhagic, spleen and liver enlarged. Also virulent to guinea-pigs which die in twelve to thirty-six hours from subcutaneous inoculation and show at autopsy edema and punctiform hemorrhages at site of inoculation, enlarged and hyperemic lymphatic glands and enlarged spleen, liver, and kidneys. Bacteria-free filtrates of seven-day cultures also toxic to guinea-pigs and white mice.

Organisms also virulent for rabbits, mice, pigeons, fish (pike and sturgeons), and crabs.

Vénulet and Padlewski: Centralbl. f. Bakteriol. [etc.], 1913, lxxi, 343.

### COCCOBACILLUS FETIDUS OZÆNÆ Perez

Described originally from ozena by Perez[1] in Buenos Aires. Reported in the United States especially by Ward.[2]

**Morphology.**—Small coccobacilli growing in long threads on agar. Non-motile. The length may be one and one-half to four times the breadth.

**Staining Reactions.**—Stained easily by the usual anilin dyes. Gram-negative.

**Cultivated** from the exudate in ozena on artificial media at 37° C. as an aërobe or facultative anaërobe. Growth slow at 20° C.

*Agar Slant.*—Smooth, glistening, abundant growth with transparent borders, not extending to the walls of the tubes. Faint yellow-brown streaks may appear in old cultures (Ward). No odor.

*Agar Stab.*—Surface growth slight. Moderate growth along line of inoculation.

*Agar Plates.*—Colonies small and opaque in the depths. Surface colonies round, at first transparent then opaque, white, or brownish, about 2 mm. in size.

*Gelatin Stab.*—Moderate growth along line of inoculation. No liquefaction. Uniform turbidity in gelatin at 37° C. without liquefaction (Ward).

*Gelatin Plates.*—Mahogany-brown lobulated colonies within a light brown zone. After four to five weeks the colonies appear as black dots on a white background.

*Potato.*—Abundant, moist, gray, or yellowish surface expansion in twenty-four hours. No gas.

*Broth.*—Turbidity with a delicate blue film on the surface of old cultures. Odor unpleasant and nauseating. Old broth cultures may have a dirty-reddish color. Proteins decomposed with the formation of indol, skatol, ammonia, hydrogen sulphid, methylmercaptan, and other by-products.

*Dunboin's Solution.*—Peptones decomposed with the formation of indol, skatol, ammonia, hydrogen sulphid, methylmercaptan, and other by-products.

*Blood-serum.*—Growth like that on agar but less abundant.

*Milk.*—Slight acidity. No coagulation. Reaction slowly becomes alkaline.

*Glucose Broth.*—Acidity and gas production. Complete reduction of indicators like litmus, methylene-blue or neutral red. Gas formula $\frac{H}{CO_2} = \frac{3}{2}$.

*Urine.*—Ammoniacal fermentation.

**Pathogenic** to rabbits, guinea-pigs, white rats, and white mice. Subcutaneous injection causes fetid abscesses. Intraperitoneal inoculation leads to the death of guinea-pigs and rabbits with extensive peritonitis. Intravenous inoculation in rabbits is followed by increased secretion from the nose, this later becoming mucopurulent. The animals may die in forty-eight hours or develop a chronic disease. Some rabbits are immune. Specific agglutinins follow the introduction of killed cultures in rabbits.

1. Perez: Ann. d. l'Inst. Pasteur, 1899, xiii, 937.
2. Ward: J. Infect. Dis., 1916, xix, 153. 1917, xxi, 338.

## FAMILY VII. BACILLACEÆ Fischer

Rods producing endospores, usually Gram-positive. Flagella when present peritrichic. Often decompose protein media actively through the agency of enzymes.

### GENUS 1. BACILLUS Cohn

Aërobic forms. Mostly saprophytes. Liquefy gelatin. Often occur in long threads and form rhizoid colonies. Form of rod usually not greatly changed at sporulation. Type species *Bacillus subtilis* (Ehrenberg) Cohn.

The aërobic spore-bearing organisms may be most conveniently classified by their size, and the size, shape, and location of their spores. The following groups may be established:

A. *Non-pathogenic*

GROUP I

Small organisms measuring 0.4 to 0.5 μ in width and 2 to 4 μ in length with small central or excentric oval spores about 0.5 μ in width and 1.25 μ in length.

*Bacillus subtilis* (Ehrenberg) Cohn.
" *subtilis* var. *viscosus* Chester.
" *vulgatus* (Flügge) Trevisan.
" *mesentericus* (Flügge) Migula.
" *pumilis* Gottheil.
" *aterrimus* Lehmann and Neumann.
" *Globigii* Migula.
" *niger* (Gorini) Migula.
" *mesentericus* var. *flavus* Laubach.
" *panis* (Vogel) Migula.

GROUP II

Medium-sized organisms measuring 0.5 to 0.75 μ in width and 1 to 3 μ in length, with central or excentric cylindrical spores, 0.5 to 0.75 μ in width and 1 to 1.5 μ in length.

*Bacillus cohærens* Gottheil.
" *simplex* Gottheil.
" *agri* Laubach and Rice.
" *tritus* Batchelor.
" *lautus* Batchelor.

GROUP III

Large organisms with square ends, growing usually in long chains, measuring about 0.5 μ in width and 3 to 6 μ in length. Spores central or excentric, round, oval, or cylindrical, measuring 0.75 to 1 μ in width and 1 to 2 μ in length.

*Bacillus mycoides* Flügge.
" *Prausnitzii* Trevisan.
" *adhærens* Laubach.
" *badius* Batchelor.

GROUP IV

Large organisms with round ends measuring 0.75 μ in width and 2.5 to 4 μ in length. Spores central or excentric, cylindrical, measuring 0.5 to 0.75 μ in width and 1 to 1.5 μ in length.

*Bacillus cereus* Frankland.
" *cereus* var. *fluorescens* Laubach.
" *albolactis* Migula.

GROUP V

Very large organisms measuring 0.75 to 1.25 μ in width and 3 to 9 μ in length. Spores central, excentric, or subterminal, measuring 0.75 to 1.25 μ in width and 1.5 to 2 μ in length; they vary greatly in shape and may be round, rectangular or reniform.

*Bacillus megatherium* De Bary.
" *petasites* Gottheil.
" *tumescens* Zopf.
" *graveolens* Gottheil.
" *ruminatus* Gottheil.
" *flexus* Batchelor.

GROUP VI

Small organisms measuring 0.5 to 0.75 $\mu$ in width and 1.5 to 3 $\mu$ in length. Spores subterminal or terminal, round, thicker than the vegetative rods, measuring about 1 to 1.5 $\mu$ in diameter.

*Bacillus pseudotetanicus* (Kruse) Migula.
" *fusiformis* Gottheil.

GROUP VII

Small organisms measuring 0.4 to 0.5 $\mu$ in width and 2.5 to 4 $\mu$ in length. Spores terminal, cylindrical, measuring 0.75 $\mu$ in width by 1 to 1.5 $\mu$ in length.

*Bacillus circulans* Jordan.
" *brevis* Migula.
" *terminalis* Migula.

GROUP VIII

Large organisms with pointed ends, measuring 0.3 to 0.5 $\mu$ in width by 1.25 to 4 $\mu$ in length. Spores develop in the center of the rods which thus become spindle-shaped. Spores large, cylindrical, measuring 0.6 to 0.8 $\mu$ in width and 0.8 to 1.25 $\mu$ in length.

*Bacillus centrosporus* Ford.
" *laterosporus* Laubach.
" *fusus* Batchelor.

GROUP IX

Producing gas from carbohydrates.
*Bacillus asterosporus* Meyer.

GROUP X

Pigment-producing.
*Bacillus cyanogenes* Hueppe.

B. *Pathogenic*

*Bacillus anthracis* Koch.
" *anthracoides* Hueppe and Wood.
" *aërobius sepis* Legros and Lecène.
" *piliformis* Tyzzer.

**BACILLUS SUBTILIS (Ehrenberg) Cohn**

Described originally by Ehrenberg[1] as *Vibrio subtilis*, subsequently by Cohn[2] as *Bacillus subtilis*. Common in soil, dust, water, milk, and in the dejecta of man and animals. Present description from Chester[3] and Lawrence and Ford.[4]

**Morphology.**—Small, thin, homogeneous bacilli measuring 0.4 by 1.5 to 2.5 $\mu$ in twenty-four-hour agar cultures. Somewhat thicker and longer on glucose agar measuring 0.5 by 1.5 to 4 $\mu$. Does not usually form threads, but occasionally they are formed in very young cultures.

Fig. 126.—*Bacillus subtilis* from six-hour culture on plain agar, stained by Gram.

Fig. 127.—*Bacillus subtilis* from forty-eight-hour culture on plain agar, stained with gentian-violet.

**Motility.**—Sluggishly motile in young cultures. Flagella peritrichous.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—Spores are formed early, appearing within twenty-four hours on plain and glucose agar. They arise in the center or toward one end of the rods and are

slightly greater in diameter than the rods, thus causing a distinct bulging. The free spores may retain bits of protplasm at each end, often unequal in amount, giving the spore a characteristic appearance. The spores rapidly lose their protoplasm, become more oval and measure about 0.5 by 0.75 $\mu$.

**Cultivation.**—Grows readily and luxuriantly on nearly all media. Optimum temperature 37° to 40° C. Growth also at lower temperatures, down to 10° C., and at higher temperatures, up to about 50° C.

*Agar Slant.*—Weakly refractive, glassy, membranous growth along line of inoculation, later spreading out over entire surface of agar. The surface is usually dry and hard, but in old cultures it becomes soft and smeary, but is always firmly attached to the agar from which it cannot be scraped off.

*Agar Stab.*—Little growth along the line of inoculation but a spreading, dry, membranous growth on the surface of the agar extending to the wall of the tube.

*Agar Plates.*—Surface colonies weakly refractive, spreading concentrically or in ameboid fashion from small dense nuclei. Under the low power edges may be complete or finely

Fig. 128.—*Bacillus subtilis* from nine-day culture on plain agar, stained with gentian-violet.

crenate. If water of condensation be present one or two colonies frequently overgrow the entire plate. Under the lower power the colonies are homogeneous and granular or irregular and gyrose. The deep colonies are punctiform and under the lower power lichen-like with irregular margins, mycelioid in character. The colonies are usually membranous, dry, hard, and glassy, and can be separated from the agar only with great difficulty.

*Glucose Litmus Agar Slant.*—Highly refractive growth, verrucose or vesicular, with milky liquid in vesicles, not spreading. Parts of growth show distinct red pigment. Acid is produced in twenty-four hours, but is replaced by alkali in about ten days, medium turning deep blue.

*Litmus Glucose Agar Plates.*—Irregular, spreading, bizarre surface colonies, usually more luxuriant than plain agar colonies. Under low power, irregular with entire edges, or fuzzy with mycelioid outgrowths from dense central nuclei. Deep colonies slightly irregular or punctiform. Under low power irregular and mycelioid with filamentous edges. Medium first acidified then made alkaline.

*Gelatin Stab.*—Slow growth along line of inoculation and rather slow cup-shaped surface liquefaction with scum production.

*Gelatin Plates.*—Surface colonies round, homogeneous, spreading, thin, and granular. Deep colonies yellowish-brown, highly refractive. Under low power granular. Colonies may also show dense central nuclei and thin mycelioid filamentous growth extending in every direction through the medium. Gelatin liquefied.

*Broth.*—Single isolated pellicles appear on the surface in twenty-four hours. In forty-eight hours these unite to form a thin branching scum which gradually becomes more dense and tough. Medium grows turbid in first twenty-four hours, but later clears. Scum is precipitated as a whole in about ten days. This manner of scum formation is characteristic of *Bacillus subtilis*.

*Peptone.*—Turbidity in the first twenty-four hours and gradual clearing with a flocculent precipitate. Scum on surface formed in the same manner as on broth, but not so dense or tough. The pellicles often show chains and branching figures. Frequently the scum has a delicate pink color after about five days' growth.

*Potato.*—Growth on potato characteristic. It is luxuriant and warty, having the appearance of many large and small dew-drops scattered along the line of inoculation. In forty-eight hours a pink pigment collects on top of this growth and persists. In older cultures a decided rose-red line in the substance of the potato marks the limit of the growth. In ten days the vesicles dry down and only a reddish-brown dry growth remains on the discolored medium. Later the growth is moist and sticky.

*Litmus Milk.*—No change in twenty-four hours and sometimes none in forty-eight hours except that the milk becomes more alkaline. In three days the medium begins to clear from the surface, the deeper parts remaining unchanged. Clearing progresses slowly, the supernatant fluid persisting as a grayish, pinkish, or yellowish muddy medium. After a month at room temperature the medium may become very alkaline and turn deep blue-purple. Milk never coagulates.

*Blood-serum.*—Vesicular dew-drop growth with pink color often very marked in twenty-four hours. Vesicles dry down, eventually leaving a hard, wrinkled growth. Medium is not usually liquefied, but according to Savage[6] it may be.

**Fermentation Tubes.**—*Glucose.*—Turbidity in bowl and arm. Scum formation like that seen in broth. Highly acid.

*Saccharose.*—Turbidity in bowl and arm with a fragile scum forming from pellicles in about two days. Acid-production, but not so marked as in glucose.

*Lactose.*—Turbidity in bowl and extending up in arm to the level of the medium in the bowl. Rest of the arm clear. Dense tough scum. Reaction alkaline.

**Thermal Death-point.**—Spores survive steaming one and a quarter hours in the Arnold sterilizer. They survive autoclaving up to and including 19 pounds pressure, but are usually destroyed by 20 pounds pressure.

1. Ehrenberg: Infusionstierschen als volkommene Organismen. Leipzig, 1838.
2. Cohn: Beiträge zur Biologie der Pflanzen. 1872, 1, 175.
3. Chester: Fifteenth An. Rep. Del. Col. Agr. Exp. Sta., 1903.
4. Lawrence and Ford: J. Bact., 1916, i, 273.

### BACILLUS SUBTILIS VAR. VISCOSUS Chester

Described originally by Chester.[1] Subsequently found by Laubach[2] in water. Morphologically identical with *Bacillus subtilis*. Characterized by the production of viscid substance in cultures, as a result of which the cultural reactions are considerably modified. The growth on the surface of media is glairy, glistening, and intensely viscous. *Gelatin* is liquefied and *blood-serum* softened. *Milk* is rapidly peptonized. Lisk[3] finds that milk shows an increase of ammonia and amino-nitrogen, but no indol, hydrogen sulphid, or mercaptan. Acid production in *dextrose* and *saccharose*, not in *lactose*. Spores survive 15 pounds in the autoclave but are destroyed by 20 pounds pressure. Killed within an hour by steam.

1. Chester: Fifteenth Ann. Rep. Del. Col. Agr. Exp. Sta., 1903.
2. Laubach: J. Bact., 1916, i, 493.
3. Lisk: J. Bact., 1924, ix, 1.

### BACILLUS VULGATUS (Flügge) Trevisan

Described originally by Flügge[1] as *Bacillus mesentericus vulgatus*. Named *Bacillus vulgatus* by Trevisan[2] and *Bacillus vulgatus* (Flügge) by Migula.[3] Common in soil, dust, water, milk, and in the dejecta of man and animals. Almost as widely distributed as *Bacillus subtilis*. Usually known as the "potato bacillus." Present description from Lawrence and Ford.

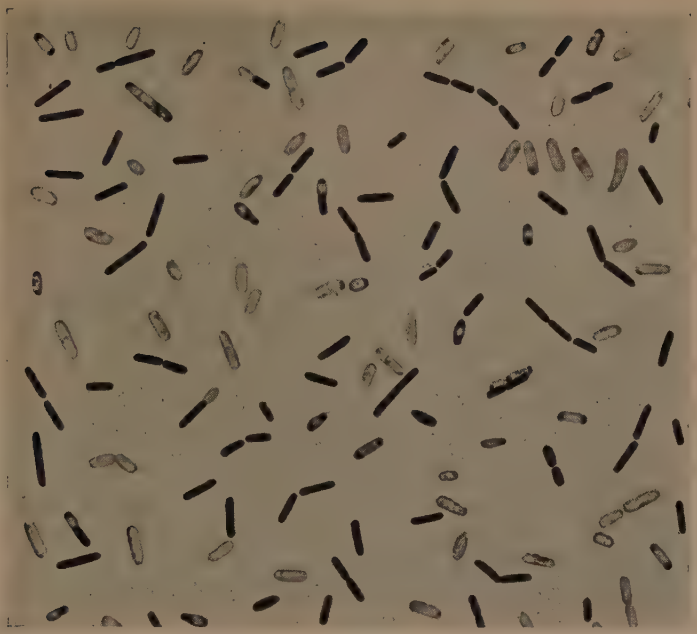


Fig. 129.—*Bacillus subtilis viscosus* from forty-eight-hour culture on plain agar, stained with gentian-violet.

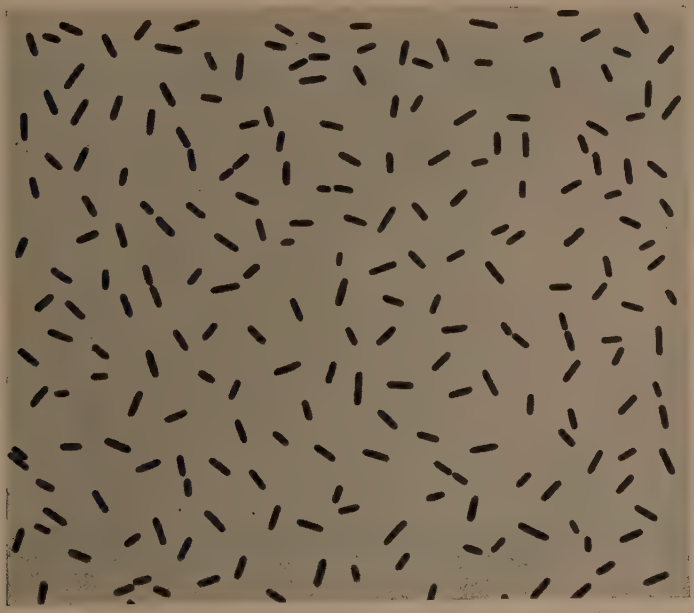


Fig. 130.—*Bacillus mesentericus vulgatus* from six-hour culture on plain agar, stained by Gram.

Fig. 131.—*Bacillus mesentericus vulgatus* from twenty-four-hour culture on plain agar, stained by Gram.

Fig. 132.—*Bacillus mesentericus vulgatus* from eight-day culture on plain agar, stained with gentian-violet.

**Morphology.**—Small homogeneous organisms usually distinctly larger than *Bacillus subtilis*, measuring 0.5 by 2 or 3 $\mu$. Occasionally short forms measuring 1.125 $\mu$ and long forms measuring 4 $\mu$ are seen on plain agar. On glucose agar the organisms are thicker and much longer measuring often nearly 0.75 $\mu$ in thickness and 5 $\mu$ in length. No tendency to the formation of threads.

**Motility.**—Active, progressive, and rotatory motility in young cultures. Flagella peritrichous.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—Spores are formed early appearing in twenty-four hours on plain and glucose agar. They arise in the center, or toward one end of the rods, but do not ordinarily bulge the rods appreciably. When free they are elongated and flattened and retain tags of protoplasm at each end. At times the protoplasm at one end is greater in amount than at the other. Such spores measure about 0.5 by 1.125 $\mu$. As they lose their protoplasm they become cylindrical, measuring about 0.5 by 1 $\mu$. In general the spores are about the same width as the vegetative rods or only slightly wider.

**Cultural Characters.**—*Agar Slant.*—Moist, profuse, thick growth on agar, easily lifted or brushed from the surface of the medium with the platinum wire. Growth is usually

Fig. 133.—Encapsulated forms of *Bacillus mesentericus vulgatus.*

white or cream-white, spreads but little from the line of inoculation and is whitest at the edge where it is heaped-up. When the water of condensation washes over the agar many small, round colonies develop apart from the main growth. In some strains the agar growth is dry and fine wrinkles develop, but the growth can always be lifted from the agar with a platinum loop.

*Agar Stab.*—Little growth along line of inoculation but rather dry, wrinkled, rooty growth spreads over the surface of the agar.

*Agar Plates.*—Surface colonies round, waxy, highly refractive, or spreading and ameboid, with greatest refraction at the edge of the advancing growth, where colonies are thickest. Under low power of the microscope the edges are entire. Deep colonies punctiform, round or elliptical. Under low power they are irregular, brown, slightly granular with entire or fuzzy edges.

*Litmus Glucose Agar Slant.*—Characteristic appearance. Luxuriant, dry, brown, and abundantly wrinkled growth develops within twenty-four to forty-eight hours. The medium is acidified. After a few days the growth usually becomes moist and the wrinkles are obliterated while the medium becomes alkaline and turns deep blue.

*Litmus Glucose Agar Plates.*—Superficial colonies are thick, highly refractive, waxy, with entire edges or spreading with irregular edges. They soon become dry and wrinkled

in the center. Under lower power opaque with entire edges. Deep colonies are punctiform, round, oval or irregular with crenated margins. Under low power opaque with irregular margins. Medium acidified at first, then turned alkaline.

*Gelatin Stab.*—Cup-shaped and surface liquefaction with heavy scum production.

*Gelatin Plates.*—Colonies round with highly refractive centers, occasionally showing beautiful concentric rings. Under the low power the colonies have a granular appearance. Medium liquefied.

*Broth.*—Turbidity within twenty-four hours and scum formation usually on second day. Medium gradually clears.

*Peptone.*—Turbidity within twenty-four hours. Thin, fragile scum after the lapse of several days. Medium gradually clears.

*Potato.*—Characteristic appearance. Thick, white, gray or pink folds, or wrinkles are formed within twenty-four to forty-eight hours, often covering the entire cut surface of the potato. Later these folds dry down to a brown, reticulate mass. Potato usually discolored.

*Litmus Milk.*—Slight clearing of the milk just beneath the cream layer usually appears in twenty-four hours. This reaction rapidly intensifies with the production of a clear fluid colored deep China-blue or purple. No acid production or coagulation. As the milk gets older complete peptonization occurs with the formation of a clear amber-colored fluid. Lisk[4] has shown that species shows an increase in ammonia and amino-nitrogen in milk, traces of acetic acid, some hydrogen sulphid, but no indol or mercaptan.

*Blood-serum.*—Thin, dry, abundant growth, usually smooth but sometimes wrinkled and pink. Growth later becomes moist and gives a suggestion of liquefaction.

**Fermentation Tubes.**—*Glucose.*—Luxuriant growth in bulb gradually extending into the closed arm. An abundant scum is formed which may be quite wrinkled. Reaction acid.

*Saccharose.*—Growth luxuriant in bowl but scanty in arm. Very thin scum may be formed after several days, but this may be lacking. Reaction varies from slight to marked acidity.

*Lactose.*—Abundant growth in bowl with late scum production. No growth in closed arm. Reaction alkaline.

**Thermal Death-point.**—Organisms survive heating in broth in the Arnold sterilizer for one hour. Survive autoclaving up to and including 19 pounds pressure, but destroyed by 20 pounds pressure.

1. Flügge: Die Mikroorganismen. 1886.
2. Trevisan: Genera.
3. Migula: p. 556.
4. Lisk: J. Bact., 1924, ix, 1.
See Peterson and Anderson, J. Bact., 1923, viii, 277.

### BACILLUS MESENTERICUS (Flügge) Migula

This organism was first described by Flügge[1] as a species distinct from *Bacillus mesentericus vulgatus* and called *Bacillus mesentericus fuscus.* Named by Migula.[2] It is one of the less common of the aërobic spore-bearing bacteria but occurs in milk, soil, dust, and water. Present description from Chester[3] and from Lawrence and Ford.[4]

**Morphology.**—Organisms about the same in morphology as *Bacillus mesentericus vulgatus.* On agar cultures in twenty-four hours homogeneous rods measuring 0.5 by 1.5 to 3 $\mu$. Sometimes shorter forms predominate in the cultures, a little over a micron in length. On glucose agar they are thicker and longer measuring 0.75 by 2 to 5 $\mu$, with many long forms measuring 6 to 8 $\mu$ in length.

**Motility.**—Active motility, progressive and rotatory, in young cultures. Peritrichous flagella.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—Spores begin to form in twenty-four hours on plain and on glucose agar. By the end of forty-eight hours they are very abundant. They appear in the center or toward one end of the rods and do not bulge the organism appreciably. The free spores are cylindrical and may retain equal bits of protoplasm at each end or this protoplasm may be unequal in amount giving a characteristic appearance. They measure about 0.5 by 1.125$\mu$. They rapidly lose their protoplasm and become slightly more oval, measuring 0.5 by 0.75 $\mu$.

**Cultural Characters.**—*Agar Slant.*—Soft, white, or cream-white growth somewhat translucent when old, spreading but little from the line of inoculation except in the presence of water of condensation. Easily lifted from the agar. Edges of growth irregular or serrate. Growth does not become dry or wrinkled.

*Agar Stab.*—Little growth along line of puncture, luxuriant growth on surface.

*Agar Plates.*—Superficial colonies round, highly refractive with entire edges, or spreading and ameboid. Under low power opaque with crenated edges. Deep colonies round and regular. Under low power slightly granular with crenated margins.

*Litmus Glucose Agar Slant.*—Thick, abundant, white or cream-white to yellow growth spreading along the line of inoculation. Medium turns acid, but as growth becomes older it again becomes alkaline and deep blue.

*Litmus Glucose Agar Plates.*—Superficial colonies round, highly refractive, with entire edges, or spreading and ameboid, with densest part of the growth along the advancing edge. Under low power of the microscope the edges are crenated. Deep colonies round or oval, under low power slightly granular with crenated margins. Medium first acidified and then turned alkaline.

*Gelatin Stab.*—Cup-shaped or surface liquefaction and scum production.

*Gelatin Plates.*—Colonies dense with liquefaction centers and granular ring at the edges of a cup-shaped liquefaction.

*Broth.*—Turbidity and a rather fragile scum appears late. Medium then clears.

*Peptone.*—Turbidity with small patches of surface growth. Medium soon clears.

*Potato.*—Growth abundant, moist, and brown with finely wrinkled or lichen-like appearance in the majority of instances. At times the fine wrinkling is lacking and only a thick, moist, brown, mealy growth is produced.

*Litmus Milk.*—Slow peptonization with the production of a lilac color turning to amber. In a few weeks digestion is complete and only a white sediment is left behind. No acidification. No coagulation. Lisk[5] has found that this organism shows an increase of ammonia and amino-nitrogen in milk, but no indol, hydrogen sulphid, or mercaptan.

*Blood-serum.*—Thin, white, dry, at times finely wrinkled growth which later becomes yellowish and moist. Suggestion of liquefaction, but this is never complete.

**Fermentation Tubes.**—*Glucose.*—Turbidity and scum in bulb and turbidity in closed arm. Reaction acid.

*Saccharose.*—Turbidity in open bulb and usually no scum. Turbidity in closed arm. Reaction acid.

*Lactose.*—Turbidity in open bulb. No scum. Arm clear. Reaction alkaline.

**Thermal Death-point.**—Spores survive one hour's heating in Arnold sterilizer and autoclaving at 19 pounds pressure. Killed by 20 pounds pressure.

1. Flügge: Die Mikroorganismen. 1886.
2. Migula: p. 555.
3. Chester: l. c.
4. Lawrence and Ford: l. c.
5. Lisk: J. Bact., 1924, ix, 1.

### BACILLUS PUMILUS Gottheil

An organism described by Gottheil in 1901 as *Bacillus pumilus* is regarded by Chester as identical with *Bacillus mesentericus.* A culture of *Bacillus pumilus* received from Kral's collection in Vienna has all the morphological, tinctorial, developmental, and cultural reactions of this species.

Gottheil: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1901, vii, 430.

### BACILLUS ATERRIMUS Lehmann and Neumann

This organism was originally described by Biel[1] and named by Lunt[2] *Bacillus mesentericus niger.* Subsequently renamed by Lehmann and Neumann.[3] It is not uncommon in milk, soil, and the intestinal contents of man. Present description from Lawrence and Ford.

**Morphology.**—Bacilli similar to *Bacillus vulgatus* in morphology. On plain agar they are homogeneous with blunt ends and measure about 0.5 by 2 to 3 $\mu$ in dimensions. On glucose agar they are thicker and longer measuring 0.75 by 2 to 4 $\mu$, but at the same time shorter forms are frequent measuring 0.75 by 1.5 $\mu$.

**Spore-formation.**—Spores are formed early, appearing in twenty-four hours on plain agar. They form in the center or toward one end of the rods which do not swell appreciably. When free they retain spurs of protoplasm at each end unequal in quantity and measure about 0.5 by 1.5 $\mu$. The spores rapidly lose their rims of protoplasm and are then oval to cylindrical, measuring 0.5 by 0.75 $\mu$.

**Motility.**—Actively motile in twenty-four-hour cultures. Flagella peritrichous.

**Staining Properties.**—Gram-positive.

**Cultural Reactions.**—This organism is identical with *Bacillus vulgatus* in all its cultural reactions except that it imparts a distinct color to the various media. This color varies from a steel-gray to a brown or black and is best seen on solid media. It is very pronounced on potato where the characteristic folds of the "*vulgatus*" are converted to thick black wrinkling bands.

**Thermal Death-point.**—The spores resist an hour's steaming in the Arnold sterilizer and 15 pounds pressure in the autoclave. They are destroyed by 16 pounds pressure.

1. Biel: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1896, ii, 137.
2. Lunt: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1896, ii, 572.
3. Lehmann and Neumann: Atlas and Principles of Bacteriology. 2. ed., Trans. by G. H. Weaver, Part II, 1901, p. 320.

### Bacillus Globigii Migula

This organism was originally described by Globig[1] as *Bacillus mesentericus ruber*.

Named by Migula.[2] Present description from Lawrence and Ford, based on a culture from Kral's collection. Organisms corresponding to this species are apparently very rare. They have been isolated in this country by Fred, Peterson, and Carroll[2] from corn mash.

**Morphology.**—Homogeneous bacilli measuring 0.5 by 2 to 3 $\mu$ in twenty-four-hour agar cultures. On glucose agar the organisms are longer and slightly thicker, often growing out into long chains, but short forms are also frequently seen.

**Spore-formation.**—Spores were formed very sparsely and at a late period in the Kral culture. They were usually seen only in sixteen to eighteen days, growth and were then characteristic mesentericus spores.

**Motility.**—Actively motile in twenty-four-hour cultures. Peritrichous flagella.

**Staining Properties.**—Gram-negative in the Kral culture. Gram-positive according to Bergey.[4]

**Cultural Characters.**—*Agar Stab.*—Slight uniform growth along line of puncture with spreading ameboid surface growth.

*Agar Plates.*—Dense, soft, white ameboid colonies similar to those of *Bacillus mesentericus*.

*Litmus Glucose Agar Slant.*—Thick, narrow, white growth along line of inoculation. The radium shows an acid reaction.

*Litmus Glucose Agar Plates.*—Thick, round, raised, soft colonies, later turning yellowish and rarely pinkish. Medium acidified.

*Gelatin Stab.*—Growth along line of inoculation and slight surface growth with liquefaction of the gelatin.

*Gelatin Plates.*—Surface colonies round, granular, punctiform with slow liquefaction. Some of the larger colonies are spreading and have a glassy surface. Deep colonies punctiform, spherical, with dense centers.

*Broth.*—Slight turbidity with no surface growth, but a flocculent precipitate. Medium is eventually turned dark yellow.

*Peptone.*—Slight turbidity with no surface growth and no precipitate.

*Potato.*—Yellow, moist growth becoming a reddish-brown. Medium is discolored.

*Milk.*—No change in forty-eight hours. In twenty days the medium shows an acid reaction with the precipitation of a white sediment.

*Blood-serum.*—Thick, transparent, spreading growth with irregular edges.

**Fermentation Tubes.**—*Glucose.*—Turbidity in open bulb. No scum. No growth in closed arm. Reaction acid.

*Saccharose.*—Turbidity. No scum. No growth in closed arm. Reaction alkaline.

*Lactose.*—Turbidity. No scum. No growth in closed arm. Reaction alkaline.

Fred, Peterson, and Carroll found that their cultures produced a pink to a deep pink color on corn mash. Starch was hydrolized, a clear zone appearing on starch plates after seventy-two hours at 28° C., 1.5 cm. in diameter. The organisms produced acid in glucose, mannose, succrose, fructose, maltose, and lactose in yeast water. The acid was first produced and then destroyed. Glucose and xylose were fermented with the production of acetone and methyl alcohol.

**Thermal Death-point.**—In the Kral culture the spores withstood one hour's sterilizing in the Arnold sterilizer, survived autoclaving at 15 pounds pressure, but were killed by 16 pounds pressure. In the cultures isolated by Fred, Peterson, and Carroll the spores were destroyed in thirteen minutes at 115° C.

1. Globig: Ztschr. f. Hyg., 1888, iii, 323.
2. Migula: p. 554.
3. Fred, Peterson, and Carroll: J. Bact., 1925, x, 97.
4. Bergey: Determinative Bacteriology. p. 300.

### BACILLUS NIGER (Gorini) Migula

This organism was first described by Gorini[1] as *Bacillus lactis niger* and is closely related to the preceding organism named by Migula.[2] The present description was worked out by Lawrence and Ford from a culture obtained from the Kral collection.

**Morphology.**—Bacilli with homogeneous protoplasm and blunt or rounded ends measuring 0.4 to 0.75 by 1.5 to 3 $\mu$ in twenty-four-hour agar cultures. No change in morphology on glucose agar.

**Spore-formation.**—Spores are formed in twenty-four hours on plain agar and in forty-eight hours on glucose agar. They appear in the center or toward one end of the rods and are oval or cylindrical in shape. The free spores may retain protoplasm at both ends and are typical of the "mesentericus" group. They measure 0.75 to 1 by 1.125 to 1.25 $\mu$ in dimensions.

**Motility.**—Active motility in young cultures. Flagella peritrichous.

**Staining Properties.**—Gram-positive.

**Cultural Reactions.**—This species has the general cultural reactions of *Bacillus mesentericus*. It grows on agar as a rather thick, moist mass with a silvery sheen, which shows black areas at the edges and in old cultures imparts a black tone to the agar. It liquefies gelatin rapidly, produces a faint acidity in milk which it first coagulates and then slowly digests. On glucose agar it tends to wrinkle slightly. It produces a faint acid in glucose, saccharose, and lactose fermentation tubes. On potato it grows as a raised brown mass and it also produces a brownish growth on blood-serum.

**Thermal Death-point.**—The spores withstand boiling one hour in the Arnold sterilizer and a pressure of 20 pounds in the autoclave. They are destroyed by a pressure of 22 pounds.

1. Gorini: Gior. d. r. Soc. ital. d'ig., 1894, xvi.
   ———: Centralbl. f. Bakteriol. [etc.], 1896, xx, 94.
2. Migula: p. 636.

### BACILLUS MESENTERICUS VAR. FLAVUS Laubach

Described by Laubach as a new variety of *Bacillus mesentericus*. Characterized by the production of an abundant yellow pigment. Common in dust and soil. Morphologically like *Bacillus mesentericus*. Growth on *agar* lemon-yellow. Slow liquifaction of *gelatin*. No change in *milk*. Slight acidity in *dextrose*, none in *saccharose* and *lactose*. Spores survive 18 pounds pressure in the autoclave and destroyed by 20 pounds. They survive one hour's steaming in the Arnold.

Laubach: J. Bact., 1916, i, 493.

### BACILLUS PANIS (Vogel) Migula

This organism was originally described by Vogel[1] from viscid bread as *Bacillus mesentericus panis viscosi* ii. Named by Migula.[2] Present description from an isolation from dust by Laubach.[3]

**Morphology.**—When first isolated this organism showed only encapsulated forms, the capsules staining readily with gentian-violet. As the organism was cultivated on artificial media in the laboratory it lost its capacity of forming easily stained capsules, but continued to manufacture a quantity of viscous material which gave a characteristic appearance to the cultures. In young cultures on plain agar, six to twenty-four hours old, the organisms are small and homogeneous with round and flattened ends, measuring 0.4 to 0.5 by 1.5 to 3 $\mu$. They show no appreciable difference in thickness on glucose agar, but tend to form long forms measuring 5 to 6 $\mu$ in length. Occasional shadow forms are seen measuring 0.75 to 1 by 1.5 to 4 $\mu$.

**Motility.**—No motility was demonstrated by Laubach. Motility described by Vogel from a single thick terminal flagellum.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—When first isolated, spores were formed early, often in twenty-four to forty-eight hours. After long cultivation in the laboratory, they appear in the cultures only after six to eight days' growth. They are formed in the centers or toward one end of the rods and are typical mesentericus spores. They retain definite rims of protoplasm, at times concentrated at one end of the spore, and measure 0.4 to 0.5 by 1 to 1.25 $\mu$. As they lose their rims of protoplasm they become more oval and measure about 0.75 by 1 $\mu$.

**Cultural Characters.**—*Agar Slant.*—Growth scanty in twenty-four hours, then becomes slightly raised, finely wrinkled, translucent, non-spreading, and viscous. When older the growth has a tendency to become somewhat dry and gray and is easily scraped off. On highly acid agar the growth is more profuse within twenty-four hours, but is not viscous.

*Agar Stab.*—Slight granular growth along the line of inoculation with occasionally a slight budding out from the stab.

*Agar Plates.*—Colonies small, drop-like, slightly irregular, showing little or no tendency to spread, glistening, elevated, and viscous. In some cases a scum-like covering which enclosed clear gelatinous material is produced about each colony.

*Glucose Litmus Agar Slant.*—Moderate non-spreading growth within twenty-four hours. The surface of the growth has a sort of honeycomb appearance caused by fine interlacing wrinkles. It is also viscous and is somewhat bluish-gray in color. The reaction is definitely acid within twenty-four hours. This acidity is followed by a reduction of the litmus and a gradual return to alkalinity.

*Glucose Litmus Agar Plates.*—The colonies correspond closely to those on plain agar, but usually attain somewhat greater dimensions.

*Gelatin Stab.*—Rapid funnel-like progressive liquefaction. Complete liquefaction results with the formation of a tenacious grayish scum.

*Gelatin Plates.*—The colonies on gelatin plates rest in cup-like excavations caused by rapid liquefaction. A definite brown center with a surrounding grayish granular area is evident in each colony under the low power of the microscope.

*Broth.*—A slight turbidity is produced within twenty-four hours with the beginning formation of a scum. The scum later is finely granular and is formed of discrete colonies. The medium clears itself by sedimentation.

*Peptone.*—Growth the same as that in broth.

*Potato.*—Growth finely wrinkled, grayish, and viscous. The wrinkles appear wave-like. When older the expansion loses its viscosity and becomes dry and granular.

*Litmus Milk.*—A gradual clearing from the top due to progressive peptonization occurs within twenty-four hours. Within forty-eight hours peptonization is generally completed. The remaining fluid has a port-wine color, but becomes amber-colored after a variable period.

*Blood-serum.*—Smooth, moist, glistening, and viscous growth. Within twenty-four hours some solution of the medium occurs along the line of inoculation (a trough-like excavation). The medium may be entirely dissolved within two weeks and a tenacious scum may be formed.

**Fermentation Tubes.**—*Glucose.*—Finely granular scum is formed which generally breaks up into large flakes. A flocculent growth is present in the bowl. The reaction is definitely acid.

*Saccharose.*—The growth is the same and the reaction also acid.

*Lactose.*—The growth is also abundant with more pronounced scum-formation. Reaction alkaline.

**Thermal Death-point.**—The spores survive 10 pounds in the autoclave, but are destroyed by 15 pounds pressure. They survive thirty minutes steaming in the Arnold, but are destroyed by one hour's exposure.

1. Vogel: Ztschr. f. Hyg., 1897, xxvi, 404.
2. Migula: p. 576.
3. Laubach: J. Bact., 1916, i, 493.

### BACILLUS COHÆRENS Gottheil

Described originally by Gottheil. Rarely found in soil, milk, and in the intestinal contents of animals. Present description from Lawrence and Ford.

**Morphology.**—Small, rather uniform, homogeneous organisms with rounded ends, measuring 0.4 to 0.6 $\mu$ in breadth and 0.75 to 2.25 $\mu$ in length in twenty-four-hour cultures on plain agar. On glucose agar the bacilli are thicker and longer measuring 0.6 to 0.75 by 2 to 5 $\mu$. On both media shadow forms appear early, often in twenty-four hours. These are made up of faintly staining protoplasm with deeply staining particles in various positions: at the ends, toward the center, and at the periphery.

Fig. 134.—*Bacillus cohærens* from six-hour culture on plain agar, stained with gentian-violet.

Fig. 135.—*Bacillus cohærens* from eight-day culture on plain agar, stained with gentian-violet.

**Motility.**—Actively motile in twenty-four-hour cultures. Flagella peritrichous.
**Staining Properties.**—Gram-positive.
**Spore-formation.**—Spores formed in two to ten days, oval or elliptical, arising in the

centers or toward the ends of the rods, which are slightly bulged on sporulation. The free spores are very delicate and stain with difficulty. They measure about 0.5 by 0.75 $\mu$. They may retain distinct rims of protoplasm, measuring 0.75 by 1.5 $\mu$. Later they lose their protoplasm and became more oval. Rarely the spores retain unequal bits of protoplasm at the ends and then they resemble the mesenteric spores slightly.

**Cultural Characters.**—*Agar Slant.*—Thin, soft, spreading, whitish growth, later becoming yellow. Easily scraped off the agar.

*Agar Stab.*—Faint growth along the line of inoculation and spreading on the surface, thick, and whitish in old cultures.

*Agar Plates.*—Surface colonies round or bizarre, thick, white. Under low power granular with dense central nuclei. Edges entire. Deep colonies punctiform. Under low power irregular with entire edges.

*Litmus Glucose Agar.*—Thick, soft, whitish growth along line of inoculation, becoming yellowish, and irregularly heaped up. Medium quite markedly acidified.

*Litmus Glucose Agar Plates.*—Surface colonies round or irregular, thick, whitish. Under low power granular and frequently showing dense central nuclei with thin peripheries and regular edges. Deep colonies punctiform. Under low power irregular with irregular edges. Reaction of medium acid.

*Gelatin Stab.*—Faint growth along line of inoculation with surface liquefaction and scum production.

*Gelatin Plates.*—Thin, circular colonies, under low power granular.

*Broth.*—Turbidity at first, then the medium clears and a dense surface growth appears which shows many clear, globular masses like globules of fat floating on the surface.

*Peptone.*—Turbidity with a faint, fragile scum.

*Potato.*—Thin, spreading, moist, yellow growth.

*Litmus Milk.*—Slow decolorization of the litmus with peptonization and the production of an amber-colored fluid.

*Blood-serum.*—Thin, whitish growth. No liquefaction. The expansion may appear finely wrinkled.

**Fermentation Tubes.**—*Glucose.*—Turbidity in bowl with surface growth and flocculent precipitate. Arm clear. Reaction acid at the end of two to three days.

*Saccharose.*—Turbidity in bowl with slight surface growth. Arm clear. Acidity at the end of two to three days.

*Lactose.*—Turbidity in bowl with very slight surface growth. Arm clear. Reaction alkaline.

**Thermal Death-point.**—In some strains the spores survive one hour's steaming in the Arnold sterilizer, and withstand 18 pounds pressure. In other isolations the spores survive 14 pounds pressure in the autoclave, but are killed by 16 pounds. They survive one hour's steaming in the Arnold.

Gottheil: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1901, vii, 430.

### BACILLUS SIMPLEX Gottheil

This organism was described by Gottheil[1] as a distinct species. The present description applies to a culture from the Kral collection and to an organism obtained from soil by Laubach. The species is evidently one of the rare spore-bearing organisms.

**Morphology.**—In the Kral culture the organisms are large homogeneous rods with rounded ends, measuring usually 0.5 to 0.75 by 3 to 4.5 $\mu$. At times much thicker forms are seen approximating 1.125 $\mu$ in thickness while longer forms, 6 $\mu$ in length, are not uncommon. The organisms often grow out into long threads or filaments 10 to 12 $\mu$ in length, especially on glucose agar. Even in young cultures the homogeneous rods lose their protoplasm and are converted into peculiar shadow forms. They are made up of a very faintly staining protoplasm in which denser aggregations of cytoplasm appear. Such forms measure 1.1 to 1.2 $\mu$ by 12 to 15 $\mu$ in dimensions. On glucose agar the organisms have the same morphology, but may show an abundance of shadow forms. Involution and shadow forms are very abundant in old cultures. In Laubach's isolation the organisms while somewhat smaller, did not differ appreciably in morphology, measuring 0.5 to 0.6 by 1.5 to 2.5 $\mu$, but also showing both the thicker and longer forms seen in the Kral culture and the characteristic shadow and involution forms. Long forms were also very common on glucose agar.

**Motility.**—Actively motile in young cultures. Flagella peritrichous.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—In the Kral culture the spores were at first formed very slowly, appearing only after the lapse of fifteen to sixteen days. Subsequently after repeated transfers spore-formation became more active and spores were often formed in twenty-four hours.

They appeared in the centers or toward one end of the rods, were no thicker than the rods from which they sprung, and were cylindrical or almost rectangular in shape. They retained rather thick walls of protoplasm for some time and measured 0.5 by 1.125 to 1.25 μ. In Laubach's isolation the spores were formed in forty-eight to seventy-two hours in the same way as in the Kral culture but were a trifle smaller, measuring 0.3 to 0.5 by 0.75 to 1 μ.

**Cultural Characters.**—*Agar Slant.*—Thin, translucent, slightly yellowish gelatinous growth, gradually becoming denser and developing occasionally a dry, slightly wrinkled surface. Single accessory colonies not uncommon at the edge of the main growth.

*Agar Stab.*—Slight uniform growth along line of puncture with a thick circular surface growth.

*Agar Plates.*—Surface colonies thin, translucent, and ameboid, developing from pin-point centers. Under low power granular. Deep colonies round or oval, regular, granular, with clean or rarely irregularly fuzzy edges.

*Litmus Glucose Agar.*—Thick, abundant, yellowish-white, heaped-up growth with serrated margins, Medium faintly acidified in old cultures.

*Litmus Glucose Agar Plates.*—Superficial colonies thin, smooth, white, and soft. Under low power granular, edges irregular, but entire. Deep colonies punctiform. Under low power irregular with irregular, rarely fuzzy margins. A trace of acid usually produced.

*Gelatin Stab.*—Faint growth along line of inoculation with cup-shaped surface liquefaction.

*Gelatin Plates.*—Round, thick, whitish colonies with concentric rings and sharply defined edges. Medium liquefied.

*Broth.*—Faint turbidity, slight sediment, no scum, but rarely a faint ring growth along side of tube.

*Litmus Milk.*—Gradual clearing with production of straw-colored fluid in the Kral culture. In Laubach's isolation a gradual clearing to a port-wine fluid. No coagulation. Later straw-colored. Lisk[2] states that *Bacillus simplex* shows an increase of ammonia and amino-nitrogen in milk, but no indol, hydrogen sulphid, or mercaptan.

*Peptone.*—Faint turbidity and sediment with rarely a slight ring growth.

*Potato.*—Thick, moist, abundant, gelatinous, yellowish-brown growth.

*Blood-serum.*—Thin, spreading, whitish growth. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Turbidity in open bulb. No scum. Arm clear. Reaction neutral or slightly acid.

*Saccharose.*—Faint turbidity in bulb. No scum. No growth in closed arm. Reaction alkaline.

*Lactose.*—Faint turbidity in bowl. No scum. No growth in closed arm. Reaction alkaline.

**Thermal Death-point.**—In the Kral culture the spores survived steaming in the Arnold sterilizer for fifteen minutes. They withstood a pressure of 15 pounds in the autoclave, but were destroyed by 16 pounds pressure. In Laubach's isolation the spores survived 10 pounds in the autoclave, but were killed by 12 pounds pressure. They survived fifteen minutes' steaming in the Arnold sterilizer, but were killed by thirty minutes' steaming.

1. Gottheil: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1901, vii, 430.
2. Lisk: J. Bact., 1924, ix, 1.

### BACILLUS AGRI Laubach and Rice

Isolated from soil by Laubach and Rice and described as a new species.

**Morphology.**—Large, uniform, homogeneous rods with round ends measuring 0.6 to 0.75 by 2.5 to 5 μ in young cultures on plain agar. Occasionally chains with a number of elements are found and shadow or transparent forms measuring 12 to 15 μ in length. On glucose agar there is no special change in morphology, but the proportion of shadow forms is greater. The morphology resembles that of *Bacillus simplex.*

**Motility.**—Active motility in young cultures.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—Spores begin to form within twenty-four hours, but are not abundant till after the lapse of three or four days. They are central or slightly excentric, no wider than the rods from which they spring. The free spores retain rims of protoplasm for some time. They measure 0.6 by 1.125 to 1.25 μ. As they lose their protoplasm they become more oval and measure 0.5 to 0.6 by 0.75 to 1.2 μ. The spore-formation resembles that of *Bacillus simplex.*

**Cultural Characters.**—*Agar Slant.*—Fairly profuse, gray, moist, smooth, glistening, opaque growth along line of inoculation with a slight tendency to spread.

*Agar Stab.*—Faint granular growth along line of inoculation.

*Agar Plates.*—Small; gray, moist, glistening, elevated colonies with opaque centers. No tendency to spread.

*Glucose Litmus Agar.*—Fairly profuse, gray, moist, smooth, glistening, opaque growth with a slight tendency to spread. The litmus is completely reduced within forty-eight hours with a very slight initial acidity which is rapidly replaced by a permanent alkalinity. The growth wrinkles slightly.

*Glucose Litmus Agar Plates.*—Grayish, moist, opaque, glistening, and slightly wrinkled colonies. The reaction is the same as that on the glucose litmus agar slant.

*Gelatin Stab.*—A definite granular growth along the line of inoculation followed by complete liquefaction. The liquefaction is funnel-like.

*Gelatin Plates.*—Definitely circumscribed, grayish, moist, glistening colonies with opaque centers and thin lace-like peripheries. The colonies rest in excavations caused by liquefaction. On examination with low power, they appear to be composed of large, flocculent masses.

*Broth.*—A very turbid growth which clears by sedimentation. No pellicle is formed.

*Peptone.*—Growth similar to that in broth.

*Potato.*—Fairly abundant, moist, heaped-up, glistening, viscous, and grayish expansion.

*Litmus Milk.*—No change within ninety-six hours, after which period a slight reduction of the litmus occurs. The reduction is completed within about seven days and then the milk is slowly peptonized. Peptonization is generally completed within fourteen days. The remaining liquid is at first grayish in color but after a variable period becomes amber-colored.

*Blood-serum.*—A fairly abundant, thin, smooth, slowly spreading, and slightly glistening brownish growth occurs. No liquefaction of the medium.

**Fermentation Tubes.**—*Glucose.*—A turbid growth occurs in the bowl and neck, but does not extend up the closed arm. No scum is formed and a very slight initial acidity is produced which gives way to a slight alkalinity.

*Saccharose.*—The growth corresponds closely to that in glucose. There is a slight alkalinity.

*Lactose.*—The growth is similar with the production of a slight alkalinity.

**Thermal Death-point.**—The spores survive 10 pounds in the autoclave, but are destroyed by 12 pounds pressure. In the Arnold they survive thirty minutes' steaming, but are destroyed by one hour's exposure.

Laubach and Rice: J. Bact., 1916, i, 493.

### BACILLUS TRITUS Batchelor

Described by Batchelor from the dejecta of children and placed in the simplex-cohærens group chiefly because of the size of the vegetative rods and the method of sporulation. It does not have all of the cultural characteristics of any other member of this group.

**Morphology.**—The morphology is best observed in forty-eight-hour agar culture where the organisms usually lie singly. They are regular in outline, with homogeneous protoplasm and round ends. They measure usually 0.75 by 3.75 $\mu$. Often short almost oval forms occur. On glucose agar the rods are larger, averaging 0.75 by 5.25 $\mu$. Whip formation occurs on plain agar and is common on glucose agar. Many of these whips measure 75 $\mu$ in length.

**Motility.**—Active progressive motility in young cultures.

**Staining Reactions.**—Gram-positive.

**Spore-formation.**—The spores are smaller than the vegetative rods. They form early, often appearing in twenty-four hours on plain and glucose agar. They are oval, measuring 1.2 by 1.5 $\mu$, and subterminal in position in the vegetative rods. The young spores retain protoplasmic tags of various lengths. As the spores mature these tags are lost, the spores become oval and then measure 0.75 by 1.125 $\mu$.

**Cultural Characters.**—*Agar Slant.*—Thick, creamy, moist, glistening growth made up in young cultures of confluent colonies, about 0.5 mm. in diameter. These gradually coalesce. Older cultures are buff in color and have a tallow-like consistency.

*Agar Stab.*—Faint growth along line of inoculation. Translucent buff growth at point of puncture.

*Agar Plates.*—Moist, opaque, round, and irregular surface colonies with dark centers and rather compact but fuzzy peripheries. Deep colonies clear cut, round, or elliptical with occasional fuzzy outlines. Under low power the colonies are reticulated, particularly in the peripheral zones.

*Litmus Glucose Agar Slant.*—Thin, dry, beaded growth in twenty-four hours with a faint acidity which does not persist.

*Litmus Glucose Agar Plates.*—Small, white, almost pin-point surface colonies. Under low power pale, round or irregular with complete edges. Deep colonies punctiform.

*Gelatin Stab.*—Faint growth along line of inoculation. No liquefaction.

*Gelatin Plates.*—Rather large, irregular, brown, homogeneous colonies with circumscribed edges. No liquefaction.

*Broth.*—Turbidity and cloudy precipitate which later becomes granular. Fragile pellicle.

*Peptone.*—Faint turbidity and precipitate. No pellicle.

*Potato.*—Faint, dry, brown growth.

*Litmus Milk.*—Faint growth in milk with practically no change in the medium. No acidity. No coagulation.

*Blood-serum.*—Shiny, wax-like, buff growth becoming pale brown. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Faint turbidity in the bulb. Slight sediment. No scum. Reaction neutral.

*Sucrose.*—Appearance the same. Reaction neutral.

*Lactose.*—Appearance the same. Reaction neutral.

**Thermal Death-point.**—Spores destroyed by 7½ pounds pressure in the autoclave. They survive after exposure for fifty-five minutes.

Batchelor: J. Bact., 1919, iv, 23.

### BACILLUS LAUTUS Batchelor

Obtained from the dejecta of children by Batchelor and placed provisionally in the simplex-cohærens group, partly because of its morphology and spore-formation and partly because of its cultural characteristics. It differs somewhat from *Bacillus simplex*, *Bacillus cohærens* and from *Bacillus agri* of Laubach and Rice.

**Morphology.**—In twenty-four- and forty-eight-hour agar cultures the vegetative rods are homogeneous with rounded ends. They vary greatly in size and shape and usually measure 1.5 $\mu$ in thickness by 2.25 to 4.5 $\mu$ in length. Shadow forms appear early with irregular aggregations of cytoplasm. They are larger than the vegetative rods, measuring 1.9 by 5.25 to 6 $\mu$. Whip forms also appear early and are very common in old cultures. Involution forms are frequent in old agar growths, taking the stain deeply and measuring 2.25 by 3 $\mu$. On glucose agar the organisms are thicker and often shorter, measuring 1.9 by 2.25 $\mu$, but longer forms predominate. Involution forms are very numerous on old glucose agar.

**Motility.**—Actively motile in young cultures.

**Staining Reactions.**—Gram-positive.

**Spore-formation.**—Spores are found early on plain agar, but later on glucose agar where they seldom appear before ninety-six hours. They are subterminal, wider than the organism from which they spring, and measure about 1.9 by 3 $\mu$. As they mature they lose their protoplasmic rims and become more oval, measuring usually 1.2 by 2.25 $\mu$.

**Cultural Characters.**—*Agar Slant.*—Very thin, dry, transparent growth without tendency to spread.

*Agar Stab.*—Very faint growth along line of puncture with a meager surface growth at point of inoculation.

*Agar Plates.*—Tiny, white colonies, round and irregular. Under low power granular with complete edges.

*Litmus Glucose Agar.*—Fairly heavy, dry, beaded growth. Reaction acid.

*Litmus Glucose Agar Plates.*—Thin, moist, irregular, and round colonies with dark centers and lighter peripheries. Under low power homogeneous and translucent with serrate edges. Acidity marked.

*Gelatin Stab.*—Faint growth along line of inoculation. No liquefication.

*Gelatin Plates.*—Small, white colonies with hazy, irregular outlines. No liquefaction.

*Broth.*—Early turbidity and late production of a hazy, rather flocculent precipitate.

*Peptone.*—Faint growth, slight turbidity, no precipitate.

*Potato.*—Faint, moist, pale brown growth.

*Litmus Milk.*—Rapid reduction of litmus, gradual development of acidity which persists. No coagulation. No peptonization.

*Blood-serum.*—Diffuse, thin, pale-brown growth with areas of yellow pigmentation. This develops into a rather dirty-brown growth in seven to eight days. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Turbidity in open bulb. No precipitate. No scum. Reaction acid.

*Sucrose.*—Turbidity in bulb. No precipitate. No scum. Reaction acid.

*Lactose.*—Turbidity in bulb. No precipitate. No scum. Reaction acid.

**Thermal Death-point.**—Spores destroyed in the autoclave at a pressure of 10 pounds. Survive 7½ pounds pressure. Destroyed by live steam in the Arnold sterilizer in thirty minutes.

Batchelor: J. Bact., 1919, iv, 23.

### Bacillus mycoides Flügge

This organism was first reported by Flügge and has since then been given other names by various authorities. It is not the same as *Bacillus ramosus liquefaciens* of Prausnitz with which it is often confused. *Bacillus mycoides* is common in milk, water, soil, and dust. Description from Lawrence and Ford.

**Morphology.**—In young cultures six to eight hours old on plain agar the organisms are homogeneous with square ends and measure usually a little more than 0.5 $\mu$ in width by 3 to 6 $\mu$ in length. They are distinctly thinner and longer than *Bacillus cereus*. As the organisms mature, the protoplasm appears more granular and a characteristic arrangement in short and long chains is seen. They then resemble the anthrax bacillus. On glucose agar the bacilli are thicker, 0.75 to 1 $\mu$, and usually about the same length. On this medium the protoplasm is converted into globular bodies which do not take the stain and which are similar to those seen in *Bacillus cereus*. These bodies may be fat. In certain instances the organisms seem to be made up of a network of fine strands in which the globular bodies hang suspended. Often the chains are curled or curved upon themselves. Old cultures show an abundance of swollen involution forms, which seem to have a skein-like arrangement

**Motility.**—Active motility in young cultures. Flagella peritrichous.

**Staining Properties.**—Gram-positive.

Fig. 136.—*Bacillus mycoides* from five-hour culture on plain agar.

**Spore-formation.**—Spores begin to form early, appearing first as small refractile bodies in the center or toward one end of the organisms, usually at the end of twenty-four to forty-eight hours. Gradually the organisms swell and the spores at the same time increase in size and at this stage a long chain of organisms, each containing a spore, may often be seen. The protoplasm soon disintegrates, leaving a rim about the spore which is round or oval or slightly rectangular. Such spores measure 0.75 to 1 by 1.125 $\mu$. Other spores are more definitely elongated and may measure 0.75 to 1 by 1 to 2 $\mu$. The spores often remain attached to each other in short or long chains. The spores vary more in size than do others of this group and may show small forms 0.4 to 0.5 by 0.5 to 1 $\mu$ and large forms measuring 1.125 by 2 $\mu$, lying side by side.

**Cultural Characters.**—*Agar Slant.*—Filamentous rhizoid growth spreading from the line of inoculation and extending into the agar. This growth is at first glassy and glistening, but later grows dull and soft. Appearance on agar characteristic.

*Agar Stab.*—Faint arborescent growth along line of inoculation with a surface development in concentric zones.

*Agar Plate.*—Surface colonies spread from dark, dense nuclei and show dense rhizoid peripheries extending into the agar on all sides. Under low power the edge of the colony is found to be composed of parallel strands of growth. Deep colonies have almost the same appearance and always exhibit the spreading peripheral mycelioid outgrowths.

*Litmus Glucose Agar.*—Thin, membranous, mycelioid growth, later becoming branched and reticulate. Growth at first moist and white, later becoming pale yellow. Medium first acidified and then turned alkaline and deep blue.

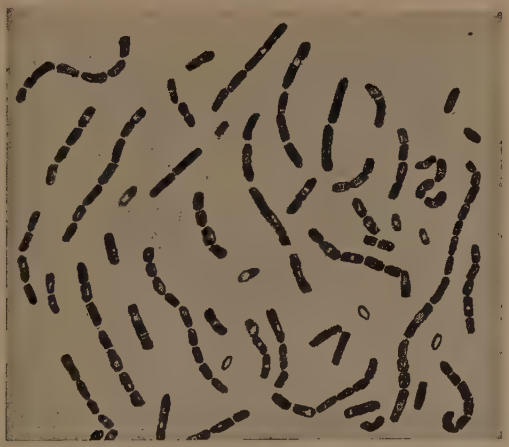


Fig. 137.—*Bacillus mycoides* from twenty-four-hour culture on plain agar.

Fig. 138.—*Bacillus mycoides* from five-day culture on plain agar.

*Litmus Glucose Agar Plates.*—Surface colonies thin, round, or irregular. Under low power found to consist of masses of matted filaments with usually dense central nuclei, from which single or parallel strands extend into the agar in every direction for long distances. Deep colonies exhibit the same small punctiform and matted mycelioid growth under lower power. Medium first acidified, and then turned alkaline.

*Gelatin Stab.*—Filamentous growth along line of inoculation with surface liquefaction.

*Gelatin Plates.*—Colonies consist of dense central nuclei with matted edges from which long strands emerge. The colonies present a peculiar appearance like a chestnut burr.

*Broth.*—No turbidity, but a flocculent suspension and a firm scum which is soon precipitated.

*Potato.*—Mealy, white expansion, later becoming brownish.

*Litmus Milk.*—Slow peptonization to an amber-colored fluid. No acidification. No coagulation.

*Blood-serum.*—Dry, mycelioid, interlacing, luxuriant growth. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Flocculent growth in bowl and arm. Scum forms and is soon precipitated. Reaction acid.

*Saccharose.*—Flocculent growth in bowl and arm. Scum is formed and precipitated. Some cultures produce moderate acidity. Others produce no acid.

*Lactose.*—Growth in open bulb with a slight extension into arm. Scum formed and soon precipitated. Reaction alkaline.

**Thermal Death-point.**—Spores survive one hour in the Arnold sterilizer and 15 pounds pressure in the autoclave. Destroyed by 16 pounds pressure.

Flügge: Die Mikroorganismen. 3. Aufl., 1896, ii, 199.

### BACILLUS PRAUSNITZII Trevisan

This organism was originally reported as *Bacillus ramosus liquefaciens* by Flügge.[1] Later correctly named by Trevisan.[2] It is sometimes regarded as identical with *Bacillus mycoides*, but a culture obtained from the Kral collection shows different reactions. Several cultures corresponding closely to the Kral culture were isolated by Laubach[2] in Baltimore from dust. The following description applies to the Kral culture and to Laubach's isolations.

**Morphology.**—In young cultures, six hours old on plain agar, the organisms are homogeneous and have round ends when free and flattened ends when in juxtaposition. They generally occur in chains of two to four elements and resemble *Bacillus mycoides* in morphology. The single cells measure 0.6 to 0.75 by 3 to 5 $\mu$. On glucose agar they are thicker, measuring 0.75 to 1 by 3 to 5 $\mu$. In older cultures, twenty-four hours, on both plain and glucose agar the protoplasm is converted into globular bodies which take the stain badly. These may be fat. They are especially abundant on glucose agar which also shows long and thick vegetative rods, measuring 0.75 to 1 by 4 to 6 $\mu$, and peculiar washed-out organisms which seem to be made up of a fine network or skein of filaments.

**Motility.**—Active motility in young cultures. Peritrichous flagella.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—Spores begin to form early, appearing in twenty-four hours on plain and on glucose agar. They are usually central, one to a cell, and are slightly wider than the vegetative rods. The organisms with spores retain their chain formation and later the free spores may also remain attached in chains. The free spores are cylindrical and measure 0.75 to 1 by 1.5 to 2 $\mu$. As they lose their protoplasm they become oval and measure 0.6 to 0.75 by 1 to 1.25 $\mu$.

**Cultural Characters.**—*Agar Slant.*—Profuse spreading dull growth, consisting of fine interlacing filaments developing from the central line of inoculation as a rhizoid mass. The early growth is extremely tenacious and extends deeply into the underlying agar. Later the growth becomes finely granular and friable and can be scraped from the medium. In general the appearance on agar is like that of a culture of *Bacillus mycoides*.

*Agar Stab.*—Abundant growth along line of inoculation and spreading surface growth.

*Agar Plates.*—Colonies consist of densely interlacing filaments spreading from opaque centers. They are dull and grayish and penetrate the agar, under the surface of which they grow in the medium.

*Glucose Litmus Agar Slant.*—Scanty growth on the surface with a pronounced acid reaction which remains permanent.

*Glucose Litmus Agar Plates.*—Colonies much the same as those on plain agar, but somewhat more profuse, with the filamentous character more pronounced.

*Gelatin Stab.*—Progressive funnel-like liquefaction often complete within three days.

*Gelatin Plates.*—Colonies consist of densely interlacing filaments spreading from opaque centers. They are dull and grayish and penetrate the gelatin, under the surface of which they grow as in agar. Each colony is soon surrounded by a zone of liquid gelatin.

*Broth.*—Granular scum and flocculent growth which soon settles to the bottom.

*Peptone.*—Similar granular scum and flocculent sediment.

*Potato.*—Viscous, yellowish-gray growth spreading profusely and rapidly over the whole medium.

*Litmus Milk.*—Reaction highly acid within twenty-four hours and a firm coagulation within forty-eight hours. Peptonization soon begins and proceeds very slowly, eventually converting the coagulum to an amber-colored fluid. Coagulation is more rapid in freshly isolated strains.

*Blood-serum.*—Profuse, moist, dull, interlacing or mycelioid growth. No peptonization.

**Fermentation Tubes.**—*Glucose.*—A flocculent growth in the bowl and a granular scum. Turbidity in closed arm. Reaction acid.

*Saccharose.*—A flocculent growth with an acid reaction. Arm usually clear.

*Lactose.*—A similar flocculent growth and an acid reaction. Arm usually clear.

**Thermal Death-point.**—Spores survive 12 pounds in the autoclave but are killed by 15 pounds. In the Arnold they survive forty-five minutes, but are destroyed by an hour's exposure.

1. Flügge: Die Mikroorganismen. 3. Aufl., 1896, ii, 201.
2. Trevisan: Genera 1889, p. 20.
3. Laubach: J. Bact.., 1916, i, 493.

### BACILLUS ADHÆRENS Laubach

Obtained by Laubach from dust. Similar organisms have been encountered frequently in soil and dust and the species is apparently widely distributed. It is characterized by the tenacity with which the growths adhere to the underlying media.

**Morphology.**—Long, slender rods with homogeneous protoplasm and flat ends, growing usually in long curved chains made up of eighteen to twenty elements. In young cultures on plain agar the individual cells measure 0.4 to 0.5 by 1.5 to 4 $\mu$. Some longer forms, 6 $\mu$ in length, may also be found. On glucose agar the organisms are homogeneous, measure 0.6 to 0.75 by 3 to 5 $\mu$, but are often longer, measuring 6 to 8 $\mu$. In older cultures (four days) many globular bodies occur on glucose agar. These may be fat. They resemble the globular bodies seen in *Bacillus cereus*.

**Motility.**—No motility ever observed, even in very young cultures.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—When first isolated this species may form spores in twenty-four hours. After long artificial cultivation spores are formed only after four to five days' growth on both plain and glucose agar. They are usually subterminal, but may be central. The rods swell appreciably before sporulation, sometimes in the center and sometimes at the ends. The free spores are oval and measure 0.6 to 0.75 by 0.8 to 1 $\mu$. They often remain fastened to each other in long chains. Frequently a bit of protoplasm remains attached to the spore which then resembles a tennis racket with its handle.

**Cultural Characters.**—*Agar Slant.*—In early growth (eighteen hours) this species slightly resembles *Bacillus mycoides*. The line of inoculation shows a distinct ridge from which fine interlacing filaments shoot out. These adhere closely to and grow into the agar. Considerable puckering of these interlacing filaments causes a roughened leathery appearance on the surface. The early growth is moist and slightly glistening, but these properties are soon lost. The edges of the growth are serrated, with little or no tendency to spread. A brownish color is found in old cultures throughout the entire medium.

*Agar Stab.*—Profuse growth along the line of inoculation and out into the medium. This has the appearance of an inverted fir-tree.

*Agar Plates.*—The colony is very characteristic. It first appears like a small colony of *Bacillus mycoides*, but within twenty-four hours the filaments seem to swell and produce a somewhat corrugated surface with a very definite elevated and yellow-brown center. The entire colony adheres closely to the agar and gradually grows into it.

*Glucose Litmus Agar Slant.*—The growth is similar to that on plain agar, but is very scanty. A definite and permanent acidity is produced.

*Glucose Litmus Agar Plates.*—Colonies considerably smaller than those on plain agar and have the same general appearance.

*Gelatin Stab.*—In gelatin growth is slow and a funnel-like liquefaction is very slowly produced.

*Gelatin Plates.*—Coarsely granular, slightly raised with definite yellow-brown centers. The centers are coarsely flocculent under the low power of the microscope. The colonies are surrounded by a slight area of liquefaction.

*Broth.*—A slight turbidity is produced and a definite scum is formed which settles to the bottom.

*Peptone.*—Growth similar to that in broth.

*Potato.*—A fairly profuse, grayish-white, moist growth. When the medium is dry the growth is scale-like.

*Litmus Milk.*—No change is noticed within twenty-four hours. After forty-eight hours a slowly progressive peptonization develops. The medium becomes amber-colored.

*Blood-serum.*—A fine-grained, leather-like growth occurs, dull gray and adherent to the medium. Later this is easily scraped off. No digestion of the serum.

**Fermentation Tubes.**—*Glucose.*—A flocculent growth occurs in the bowl and extends into the closed arm. Reaction acid.

*Saccharose.*—Turbidity in bowl. Arm clear. Reaction neutral or slightly acid.

*Lactose.*—Turbidity in bowl. Arm clear. Reaction not changed.

**Thermal Death-point.**—The spores resist 18 pounds pressure in the autoclave, but are destroyed by 20 pounds. They survive one hour's steaming in the Arnold.

Laubach: J. Bact., 1916, i, 493.

### BACILLUS BADIUS Batchelor

This organism was isolated from the dejecta of children by Batchelor. It is characterized by the beautiful arborescent colonies which it produces on agar plates and in agar stab cultures. These colonies are not unlike those of *Bacillus mycoides* and *Bacillus Prausnitzii* of the mycoides group to which also *Bacillus adhærens* of Laubach has been referred. It differs from these organisms in its cultural reactions and in its production of a brown pigment, but may be placed in the same group.

**Morphology.**—Long vegetative rods with homogeneous protoplasm and round ends, varying in length from 2.35 to 4.5 $\mu$ in twenty-four-hour cultures on plain agar. The shorter forms predominate in older cultures. Width about 0.75 $\mu$. On glucose agar the organisms are somewhat swollen. Rarely short chains are formed. Whip form not seen.

**Motility.**—Active motility in young cultures.

**Staining Reactions.**—Gram-positive.

**Spore-formation.**—Spores begin to form by the first day on glucose agar, but only after four to five days on plain agar. They are oval in shape, subterminal in position. As the sporulating rods degenerate, tags of protoplasm are left, sometimes at one end and sometimes at both ends of the spore. They measure 1.2 by 1.8 $\mu$. Older spores become free from protoplasmic tags and assume an oval contour with a tendency to squaring of the ends. Such naked spores measure about 0.75 by 1.5 $\mu$.

**Cultural Reactions.**—*Agar Slant.*—Rapidly spreading, glistening, moist growth, buff-colored in young cultures with areas of brown pigmentation. At the end of forty-eight hours the entire growth is brown. The surface remains moist and glistening and the growth is easily scraped from the agar. Later the agar itself becomes pigmented.

*Agar Stab.*—Slight growth along line of inoculation, definitely arborescent. Surface growth at point of puncture spreading and abundant.

*Agar Plates.*—Buff, opaque, moist colonies showing great irregularity in size and shape. The edges are fuzzy with beautiful arborizations. Under low power, the colonies are reticulated with fine, extensive, radial outgrowths.

*Litmus Glucose Agar.*—Rapidly spreading, dry, buff-colored growth easily scraped from the medium. No acid. Agar gradually becomes dark blue.

*Litmus Glucose Agar Plates.*—Colonies similar to those on plain agar with somewhat accentuated arborizations. Dirty-white to pale brown in color. No acidity.

*Gelatin Stab.*—Slow growth along line of inoculation with rapid cup-shaped surface liquefaction. The liquid gelatin becomes definitely brown.

*Gelatin Colonies.*—Irregular and brown. No definite arborization. The edges of the colonies are fuzzy and their texture resembles cotton. Rapid liquefaction.

*Broth.*—Rapid production of dense turbidity. No scum. No precipitate. Medium becomes very brown.

*Peptone.*—Fairly rapid growth without scum or precipitate. Medium becomes brown in color.

*Potato.*—Faint, dry, brown growth.

*Litmus Milk.*—Gradual decolorization of the litmus and clearing of the proteins in the milk. Digestion complete in about a week when the fat at the surface becomes brown from the adsorption of the pigment.

*Blood-serum.*—Dry, buff growth with some tendency to a beaded appearance. It becomes brown in two to three days. Some softening of the serum which gradually dries without definite liquefaction.

**Fermentation Tubes.**—*Glucose.*—Turbidity in bulb and neck. No scum or precipitate. Reaction neutral.

*Sucrose.*—Turbidity in bowl and neck. No scum. No precipitate. Reaction neutral.

*Lactose.*—Turbidity in bowl. No scum. No precipitate. Reaction neutral.

**Thermal Death-point.**—Spores in broth emulsion survive a pressure of $2\frac{1}{2}$ pounds in the autoclave, but are destroyed by 5 pounds pressure. They survive fifteen minutes' steaming in the Arnold, but are destroyed by thirty minutes' exposure.

Batchelor: J. Bact., 1919, iv, 23.

### BACILLUS CEREUS Frankland

This organism was discovered by the Franklands[1] in 1887. It has since been reported under a variety of names and it is impossible to say how many different species are identical with it. It is the most widely distributed aërobic spore-bearing bacillus, being found abundantly in milk, soil, dust, water, and in the intestinal contents of man and animals. It is particularly common as a laboratory contamination. The present description applies to cultures received from the Kral collection, from the American Museum, from a number of American laboratories, and to over a hundred isolations of Lawrence and Ford.

**Morphology.**—Regular bacilli with homogeneous protoplasm and rounded ends, in young cultures measuring about 0.75 by 2.25 to 4 $\mu$. Many of the organisms show peculiar refractile bodies of various sizes as the cultures get older, presenting a characteristic appearance. The nature of these bodies is not clear as they do not give reactions for starch or volutin. They can usually be differentiated from the beginning spores. On glucose agar the bacilli are thicker and longer, measuring 0.75 to 1 by 3 $\mu$. Here the entire protoplasm of the organism is converted into these bodies. They are globular, highly refractile, and are often as thick as the organism.

**Motility.**—Actively motile in young cultures. Flagella peritrichous.

**Staining Properties.**—Gram-positive.

Fig. 139.—*Bacillus cereus* from seven-hour culture on plain agar.

**Spore-formation.**—Spores are formed early on both plain and glucose agar, often appearing within twenty-four hours or even in less time. They are central in position, excentric or even subterminal, but the latter location of the spore is rare. The spores are usually wider than the organisms from which they spring and thus bulge the rods slightly. The free spores retain their protoplasm at the ends for some time, usually in equal amounts. Often, however, the protoplasm is greater at one end than at the other and the spore then has a characteristic appearance like an enlarged mesentericus spore. The free spores are cylindrical, soon shed their protoplasm and measure 0.5 to 0.75 by 1.125 to 1.5 $\mu$.

**Cultural Characters.**—*Agar Slant.*—Abundant, thick, white, mealy growth along the line of inoculation, sometimes with arborescent edges. In older cultures the growth is much thicker, yellowish-white, and may show pellucid areas surrounded by more highly refractive patches.

*Agar Stab.*—Little growth along the line of inoculation, but luxuriant surface growth, spreading over the entire surface of the agar and extending to the walls of the tube.

*Agar Plates.*—Round, raised, dense, highly refractive surface colonies. If a slight

amount of water of condensation be present the colonies may be ameboid. Under low power the colonies consist of dense central nuclei with spreading peripheries made up of numerous curling and parallel chains. The colonies are soft and easily lifted from the agar.

Fig. 140.—*Bacillus cereus* from twenty-four-hour culture on plain agar.

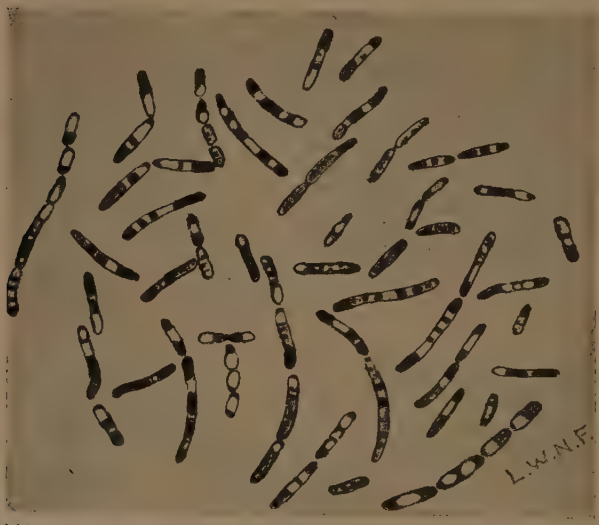



Fig. 141.—*Bacillus cereus* from twenty-four-hour culture on glucose agar.

Deep colonies punctiform, stellate or rhizoid. Under low power they are fuzzy, irregular and may resemble a chestnut burr.

*Litmus Glucose Agar Slant.*—Thick, yellowish-white growth along the line of inoculation and spreading out over the entire surface. The medium is acidified and the growth is sometimes distinctly yellow. Typical cultures rapidly decolorize the litmus and then become

alkaline and the agar turns deep blue. Occasionally the cultures are less active alkali-producers and the medium remains permanently acid. Such cultures, however, can usually be stimulated to alkali production by plating and they then give characteristic growths.

*Glucose Agar Plates.*—Surface colonies round or bizarre, heaped up, with irregular margins, smaller than plain agar colonies. Under low power granular, with dense central nuclei and irregular margins, showing fine parallel strands. Deep colonies small, irregular or round. Under low power they consist of dense central nuclei with fine, irregular, or parallel strands in the periphery.

*Gelatin Stab.*—Uniform growth along entire line of inoculation with a liquefaction also along the entire line. The liquefaction becomes cup-shaped or sacculated with a surface scum. It is rapid and frequently in two days the entire gelatin tube is liquefied.

*Gelatin Plates.*—Loosely filamentous colonies with dense, central nuclei and spreading irregular margins, often very thin with entire edges. Gelatin liquefied rapidly.

*Broth.*—Heavy turbidity in twenty-four hours with no scum except occasionally a slight ring growth. In two days a heavy friable scum is produced which is entirely precipitated within a short time. The medium gradually clears while a heavy flocculent precipitation is deposited.

*Peptone.*—Heavy turbidity in twenty-four hours. A scum appears usually on the second day and is soon precipitated. It is like that produced in broth, but is more friable.

*Potato.*—Thick, white, mealy growth in a few days turning yellowish or brown with a discoloration of the potato. This brownish growth may become very moist and slimy and is occasionally measly, but never vermiform. It never assumes an appearance similar to that seen with cultures of *Bacillus subtilis* or *Bacillus vulgatus.*

*Litmus Milk.*—With the majority of cultures peptonization begins immediately and progresses rapidly in three zones. The surface zone is amber, the middle zone violet, the lowest zone blue. Peptonization continues until the entire milk tube is converted into an amber fluid with a slight sediment. The milk does not coagulate. With some cultures the three-zone appearance does not show but the milk is gradually changed to a muddy gray-colored fluid. Eventually, however, the same clear, amber-colored fluid is produced. Lisk[2] has found *Bacillus cereus* shows an increase of ammonia and amino-nitrogen in milk with some indol production, but no hydrogen sulphid or mercaptan.

*Blood-serum.*—Thick, white, dry, smooth growth. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Abundant growth in bowl and arm. A friable scum forms which is soon precipitated. Turbidity gradually disappears and a flocculent precipitate is deposited. Reaction highly acid.

*Saccharose.*—Abundant growth in bowl and arm. A filmy and friable scum forms which is soon precipitated. In many cultures the reaction is acid. Reaction usually alkaline.

*Lactose.*—Turbidity in bowl with scum formation. Arm clear. Reaction alkaline.

**Thermal Death-point.**—The spores survive steaming one hour in the Arnold sterilizer and autoclaving at 19 pounds pressure. Killed by 20 pounds pressure.

1. Frankland, G., and Frankland, P.: Philosophical Transactions of the Royal Society of London, 178, B, 1887, p. 297.
2. Lisk: J. Bact., 1924, xi, 1.

*Note.*—Several aërobic spore-bearing organisms show pellucid areas on surface growths. This phenomenon is well recognized by workers on this group of bacteria. No adequate explanation for it has ever been suggested.

### Bacillus cereus var. fluorescens Laubach

Described by Laubach as a variety of *Bacillus cereus*, characterized by its production of a greenish-yellow fluorescence. Common in water and soil. Morphologically like *Bacillus cereus*. *Agar* and *gelatin* intensely greenish-yellow. Rapid liquefaction of *gelatin* and digestion of *milk*. Acidity in *dextrose*, not in *saccharose* or *lactose*. The spores survive 12 pounds pressure in the autoclave and are destroyed by 15 pounds. Destroyed by steaming one hour in the autoclave.

Laubach: J. Bact., 1916, i, 394.

### Bacillus albolactis Migula

This organism was apparently first obtained in pure culture in 1887 by Loeffler,[1] who found it in boiled milk which had soured and clotted and who called it *Bacillus lactis albus*. Named by Migula.[2] It is possibly identical with *Bacillus corrugatus* Migula (*Bacillus* No. II of Flügge[3]), with *Bacillus*

*bernensis* Lehmann and Neumann, and with the organism described recently by Neide as *Bacillus teres* which was also obtained from boiled milk which had subsequently soured. It is common in boiled milk and produces the souring, clotting, and subsequent peptonization seen so frequently in this material. Present description from Lawrence and Ford.

**Morphology.**—These organisms are identical morphologically with *Bacillus cereus*. In young cultures six to twenty-four hours old, on plain agar, they have round ends and measure 0.5 to 0.75 by 2.25 to 4 $\mu$. The protoplasm may be homogeneous or may show globular bodies of various dimensions. On glucose agar the globular bodies are much more abundant and give the organism a characteristic appearance. Here the rods measure 0.75 to 1 by 2.5 to 4 $\mu$.

**Motility.**—Actively motile in young cultures.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—Spores are formed readily on plain and on glucose agar. They are abundant in twenty-four to forty-eight hours and have the same appearance as the spores of *Bacillus cereus*. They are usually central or slightly excentric and a trifle wider than the organisms from which they spring, thus bulging the rods somewhat. The free spores may retain equal or unequal bits of protoplasm at the ends and have a characteristic appearance. They are oval to cylindrical and measure 0.5 to 0.75 by 1.5 to 2.2 $\mu$. The spores are frequently seen in pairs attached by their protoplasmic remnants, and also sometimes in chains.

**Cultural Characters.**—*Agar Slant.*—Luxuriant, thick, white growth with a smooth and glistening surface, spreading over the entire agar. Some cultures show a delicate transverse wrinkling.

*Agar Stab.*—Fine, slightly arborescent growth along line of inoculation. Thick, white, wrinkled surface growth.

*Agar Plates.*—Surface colonies thick, raised, round, or bizarre, frequently showing dense central nuclei. Under low power granular with dense central nuclei and spreading peripheries made up of curved parallel strands. Deep colonies small, round, or irregular. Under low power irregular, mossy, with irregular fuzzy margins.

*Litmus Glucose Agar Slant.*—Thick, yellowish-white, moist growth, spreading over the entire agar and wrinkling slightly at the base when the culture is very active. Reaction in medium acid in the first few days, but gradually alkali is produced and the agar turns dark blue.

*Litmus Glucose Agar Plates.*—Surface colonies thin, translucent, somewhat smaller than plain agar colonies. Under low power they show thin peripheries made up of curling parallel strands. Deep colonies round or irregular. Under low power irregular, mossy with irregular fuzzy margins. Medium first acidified and then made alkaline.

*Gelatin Plates.*—Surface colonies round, spreading concentrically and composed of a central loose mass of filaments denser than the surrounding zone. Deep colonies are composed of spherical masses of loose filaments with irregular, mossy, or bristling margins. Rapid liquefaction.

*Gelatin Stab.*—Growth along line of puncture with a rapid, cup-shaped liquefaction and scum production.

*Broth.*—Turbidity with ring growth in twenty-four hours and scum formation in two to three days. Scum quickly precipitated.

*Peptone.*—Turbidity with scum formation on the second day. Scum usually persists.

*Potato.*—Thick, white, moist growth, later becoming yellowish-brown. Never wrinkled or vermiform, rarely measly. Medium discolored.

*Litmus Milk.*—Acid production and coagulation, usually within twenty-four hours. The coagulum is at first firm, but gradually undergoes peptonization. Odor is distinctly fecal and very disagreeable. Lisk[4] has shown that this organism shows an increase of ammonia and amino-nitrogen in milk, but no indol, hydrogen sulphid, or mercaptan.

*Blood-serum.*—Thick, white growth. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Abundant growth in bowl extending up into closed arm which becomes turbid. Flocculent precipitate forms, but usually no scum. Reaction highly acid.

*Saccharose.*—Turbidity in bulb extending up into the closed arm. Flocculent precipitate. No scum. Reaction highly acid.

*Lactose.*—Turbidity in bowl extending up into the closed arm. No precipitate but usually a thick scum is formed. Reaction highly acid.

**Thermal Death-point.**—Organisms survive one hour in the Arnold sterilizer and autoclaving to 15 pounds pressure. Killed by 16 pounds pressure.

1. Loeffler: Berl. klin. Wchnschr., 1887, xxiv, 607.
2. Migula: p. 577.
3. Flügge: Ztschr. f. Hyg., 1894, xvii, 272.
4. Lisk: J. Bact., 1924, ix, 1.

### BACILLUS MEGATHERIUM DeBary

This organism was originally found and named by DeBary[1], and has since been described under a variety of names by a number of authors. It is one of the most common of the spore-bearing bacteria and has been found in dust, soil, milk, water, and as a laboratory contamination. The present description applies to cultures obtained from the Kral collection and from the American Museum, and to over a hundred isolations by Lawrence and Ford.

**Morphology.**—These are the largest of the spore-bearing organisms. On plain agar in young cultures, from eight to twenty-four hours old, they are long and thick, with homogeneous or slightly granular protoplasm, measuring 0.75 to 1.25 by 3 to 9 $\mu$. On glucose agar they are even thicker measuring 1.25 to 1.5 $\mu$ in width. On both media long forms occur but especially on glucose agar. These may measure 30 to 45 $\mu$ in length and may show homogeneous protoplasm without evident segmentation. The protoplasm of the organism is at first homogeneous, but by the end of twenty-four to forty-eight hours it is converted into a mass of globular bodies resistant to the stains. These globular bodies are clear, highly refractile, bulge the organisms somewhat, and are quite numerous, six to eight appearing in each rod. They thus give the organism a peculiar and characteristic appearance. They show most markedly on glucose agar, but are also present on plain agar where they can best be demonstrated by decolorizing an overstained preparation. Their nature is not clear as they do not take any special bacterial stains. Shadow or transparent forms appear in *Bacillus megatherium* early, both on plain and glucose agar. These measure 1.2 to 1.5 by 4 to 10 $\mu$, take the stain very faintly and show peculiar bodies of agglomerated protoplasm at the sides or sometimes at the ends. These transparent forms are often thicker and longer and may even measure 2 by 40 to 45 $\mu$. Occasionally they are distinctly oval with rounded ends, measuring about 1.5 by 4 $\mu$, and may show a small bunch of cytoplasm at the side. When these forms are in chains they are exactly like the original pictures of De Bary.

**Motility.**—Active, progressive and rotatory motility in young cultures. Flagella peritrichic, four to six in number (Migula[2]).

**Staining Properties.**—Gram-positive.

**Spore-formation.**—Spores are formed abundantly on plain agar in twenty-four hours and on glucose agar in forty-eight hours. They appear in the center or slightly toward one end of the rods and are usually of the same diameter, but may be slightly thicker. Sometimes two spores seem to arise in one rod, but these may possibly be in a rod just prior to division. In general each rod has a single spore. The spores occasionally lie obliquely in the rods. Frequently two spores are at opposite ends of rods lying in juxtaposition and these may remain attached in chains and present a characteristic appearance. The spores retain protoplasm at the ends for some time. When this is unequal in amount the spore has somewhat the shape of a tennis racket and handle. The free spores are oval to cylindrical and measure 0.75 to 1.2 by 1.5 to 2 $\mu$. They are often flattened on one side, having an appearance described as kidney-shaped or reniform. The spores show great variations in size, more than is usual with aërobic sporulators.

**Cultural Characters.**—*Slant Agar.*—Thick, raised, soft, white, or cream-colored growth which shows a pink tinge by reflected light, with many small, minute, pellucid areas. As the cultures get older the growth becomes pale yellow.

*Agar Stab.*—Slight growth along line of inoculation, heaped-up and spreading slightly on the surface. Later surface growth becomes slightly pinkish.

*Agar Plates.*—Surface colonies round, thick, white, or cream colored, highly refractive, turning pale yellow or yellowish-brown in old cultures. Under low power slightly granular, brownish-yellow, with entire margins. Deep colonies punctiform. Under low power round or irregular with entire edges, brown and granular.

*Litmus Glucose Agar Slant.*—Thick, luxuriant growth along line of inoculation, at first white and then pale yellow or cream-colored. Medium at first acidified but later becomes alkaline and changes from a dark blue to a smoky-brown, while the growth becomes a dark gray or smoky-brown.

*Litmus Glucose Agar Plates.*—Large, round, raised surface colonies, cream-colored to pale yellow, with heaped-up central nuclei. Under low power dark, slightly granular with entire edges. Deep colonies punctiform. Under low power dark, irregular, bizarre, with entire edges. Medium is acidified and then made alkaline.

*Gelatin Stab.*—Funnel-shaped liquefaction. No scum.

*Gelatin Plates.*—Round colonies with concentric zones of growth. Under low power cloudy central nuclei with filamentous peripheries.

*Broth.*—Turbidity, but no scum.

*Peptone.*—Turbidity, but no scum.

*Potato.*—Thick, white, mealy growth later becoming pale or cream-yellow.

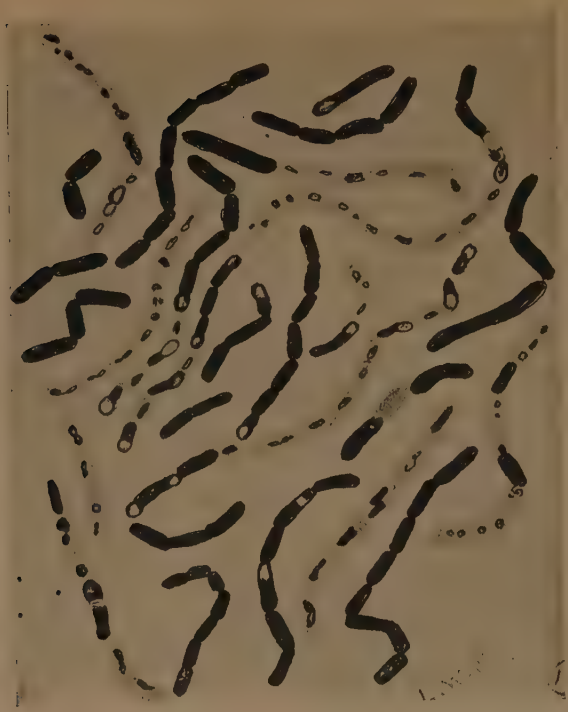


Fig. 142.—*Bacillus megatherium* from twenty-four-hour culture on plain agar.

Fig. 143.—*Bacillus megatherium* from twenty-four-hour culture on glucose agar.

*Litmus Milk.*—No change within twenty-four hours then a gradual peptonization with the production of a port-wine-colored fluid. No acidification. No coagulation. According to Lisk[3] this organism shows an increase of ammonia and amino-nitrogen in milk, but no indol, hydrogen sulphid, or mercaptan.

*Blood-serum.*—Thick, white, moist, heavy growth, cream-white to yellow in color. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

*Saccharose.*—Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

*Lactose.*—Slight turbidity in open bulb. No scum. No growth in closed arm. Reaction alkaline.

**Thermal Death-point.**—Spores withstand one hour's steaming in the Arnold sterilizer and 18 pounds pressure in the autoclave. Killed by 19 pounds pressure.

1. De Bary: Vergleichende Morphologie und Biologie der Pilze. Mycetozoen und Bakterien. 1884, p. 500. Vorlesungen über Bakterien. 2. Aufl., 1887, p. 13.
2. Migula: p. 516.
3. Lisk: J. Bact., 1924, ix, 1.

### . BACILLUS PETASITES Gottheil

This organism was reported originally by Gottheil. Its chief point of differentiation from *Bacillus megatherium* is that it produces a distinct yellow pigment on artificial media, particularly on plain agar and on potato. It is extremely common, having been found in dust, soil, water, milk, and various milk products. The present description applies to a culture from the Kral collection and to over a hundred isolations of Lawrence and Ford.

Fig. 144.—*Bacillus petasites* from seven-hour growth on plain agar.

**Morphology.**—The organisms do not differ appreciably in morphology from *Bacillus megatherium.* They are homogeneous or slightly granular rods, measuring 0.75 to 1.5 by 3 to 6 $\mu$ on plain agar in young cultures (eight to twenty-four hours), and 1 to 1.75 by 3 to 6 $\mu$ on glucose agar. Long forms measuring 12 to 25 $\mu$ are also seen on both plain and on glucose agar. Shadow forms with faintly staining protoplasm, like those seen in *Bacillus megatherium,* are common as well as the peculiar refractile globular bodies.

**Motility.**—Active, progressive, and rotatory motility in young cultures. Flagella peritrichous (Bergey).

**Staining Reactions.**—Gram-positive.

**Spore-formation.**—Spores are formed abundantly in twenty-four hours on plain and on glucose agar. The spores are oval to rectangular, of about the same width as the rods from which they spring and frequently form long chains. The free spores may retain tags of protoplasm, but soon lose them and then show great variations in size and shape. They may be nearly round, oval, rectangular and reniform and measure 0.75 to 1 by 1.5 to 2 $\mu$.

**Cultural Characters.**—*Agar Slant.*—Thick, moist, abundant, mealy growth at first pinkish by reflected light, then becoming bright lemon-yellow. Agar slightly discolored.

*Agar Stab.*—Slight growth along inoculation line, heaped-up yellowish growth on surface.

*Agar Plates.*—Surface colonies thick, white or yellow, highly refractive. Under low power dark and granular, with entire or mycelioid edges. Deep colonies punctiform. Under low power irregular, with irregular edges showing mycelioid, rooty, or fuzzy edges.

*Litmus Glucose Agar.*—Luxuriant, thick, heaped-up growth at first yellow then assuming an orange and then a dark brown color. Reaction of medium first acid, then alkaline. It eventually becomes smoky-brown.

*Litmus Glucose Agar Plates.*—Surface colonies round, regular, and thick, or thin and spreading. Under low power granular with entire edges. Deep colonies punctiform. Under low power granular, irregular, with fuzzy edges. Reaction of medium acid at first then alkaline.

*Gelatin Stab.*—Growth along line of inoculation with funnel-shaped surface liquefaction. No scum formation.

*Broth.*—Turbidity. No scum. Medium eventually becomes yellow.

*Peptone.*—Turbidity. No scum.

*Potato.*—Thick, mealy, bright yellow growth gradually becoming dark yellow.

*Litmus Milk.*—No change in twenty-four hours, then a gradual peptonization with the production of a port-wine-colored fluid.

*Blood-serum.*—Thick, dry, yellowish, moist growth becoming pale to bright yellow. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Slight turbidity in bulb. No scum. No growth in closed arm. Feeble acid production.

*Saccharose.*—Slight turbidity in bulb. No scum. No growth in closed arm. Faint acid production.

*Lactose.*—Slight turbidity in bulb. No scum. No growth in closed arm. Reaction alkaline.

**Thermal Death-point.**—Spores survive steaming in Arnold sterilizer thirty minutes, but are killed by one hour exposure. Withstand 19 pounds pressure in autoclave, but killed by 20 pounds pressure.

Gottheil: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1901, vii, p. 430.

### BACILLUS TUMESCENS Zopf

This organism was originally described by Zopf. A culture received from the Kral collection and another received from the American Museum agree in their morphological, developmental, tinctorial, and cultural features, all of which are identical with those of *Bacillus megatherium.*

Zopf: Die Spaltpilze. 3. Aufl. 1885, p. 82.

### BACILLUS GRAVEOLENS Gottheil

This organism was reported by Gottheil[1] as a new species. A culture from the Kral collection has all the cultural reactions of *Bacillus megatherium.* Morphologically it is about the same size, forms spores in the same way, is Gram-positive, produces globular bodies on plain and glucose agar and undergoes involution with the formation of shadow or washed-out forms. It shows, however, a distinct tendency to produce curved or spiral forms. On the basis of this one characteristic it is hardly justifiable to make it a distinct species. It should be noted that this use of the term "*graveolens*" is probably incorrect since a *Bacterium graveolens* was described by Bordoni-Uffreduzzi[2] in 1886. This was a small non-sporulating bacillus producing a green pigment.

1. Gottheil: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1901, vii, 430.
2. Bordoni-Uffreduzzi: Fortschritte der Medicin, 1886, p. 157.

### BACILLUS RUMINATUS Gottheil

The type "ruminatus" was first noted by Gottheil.[1] In morphology and cultural characters it closely resembles *Bacillus megatherium* from which it is distinguished by its porcelain-white growth, particularly in young cultures. The present description applies to an organism found by Laubach[2] in dust and subsequently in water which corresponds to Gottheil's original description, but not to that given by Chester. We believe that it may properly be called *Bacillus ruminatus*.

**Morphology.**—Homogeneous rods with rounded ends, measuring 0.6 by 2.5 to 4 $\mu$ in young cultures on plain agar. On glucose agar they are distinctly thicker and longer measuring 8 to 10 $\mu$. Rarely long forms are found on this medium measuring 8 to 10 $\mu$ in length. Shadow or washed-out organisms are common on both plain and glucose agar, measuring 1 to 1.5 by 3 to 5 $\mu$. The organisms often appear in short chains.

**Motility.**—Active motility in young cultures. Flagella peritrichous (Bergey).

**Staining Properties.**—Gram-positive.

**Spore-formation.**—Spores are formed early, often in forty-eight hours, especially when the strains are first isolated. They are central or slightly eccentric and on sporulation swell or bulge the organisms from which they spring. They measure usually 0.6 by 1.25 to 1.5 $\mu$. Some spores are however somewhat smaller and more oval.

**Cultural Characters.**—*Agar Slant.*—Moderate growth along line of inoculation forming a definite ridge. There is a tendency for the growth to spread to either side and the opacity of the growth decreases toward the periphery which is translucent. The growth is glistening, raised, moist, and has a pure white color which becomes more apparent with age.

*Agar Stab.*—Growth along the line of inoculation, distinctly granular.

*Agar Plates.*—The colonies are very characteristic. Some are round and regular while others show a considerable tendency to spread. They are moist, opaque, raised, glistening, and white. The majority show dense centers surrounded by thin areas which are in turn surrounded by translucent shell-like peripheries.

*Glucose Litmus Agar Slant.*—The growth is similar to that on agar, although somewhat variable as to amount. The distinct white coloration is also evident. A definite and permanent acidity is produced.

*Glucose Litmus Agar Plates.*—The colonies correspond closely to those on agar but are less profuse.

*Gelatin Stab.*—A fairly rapid progressive cone-like liquefaction occurs.

*Gelatin Plates.*—The colonies rest in cup-like excavations caused by liquefaction. They are definitely circumscribed and have an opaque center which is surrounded by a less dense grayish area. This in turn is inclosed by a more dense grayish ring. Outside this ring the opacity decreases toward the periphery.

*Broth.*—A fine fragile pellicle is formed with some turbidity. The medium clears by sedimentation.

*Peptone.*—Similar scum, but less marked turbidity.

*Potato.*—Cream-white, moist, profuse growth developing in twenty-four to forty-eight hours.

*Litmus Milk.*—Within twenty-four hours a slight acid reaction occurs and the milk often shows a distinct thickening at the bottom. The coagulation is not definite however and peptonization begins usually within forty-eight hours. As it advances a clear zone of amber-colored fluid is found at the upper part of the milk tube. This gradually increases until all the milk is peptonized.

*Blood-serum.*—Fairly profuse, moist, glistening, smooth, whitish growth. Some softening of the serum, but no definite liquefaction.

**Fermentation Tubes.**—*Glucose.*—A slight turbidity in the bowl and neck with a reduction of the litmus in the closed arm. Reaction acid.

*Saccharose.*—A similar growth with an acid reaction.

*Lactose.*—Only a slight turbidity develops with an alkaline reaction.

**Thermal Death-point.**—The spores survive 20 pounds pressure in the autoclave but are destroyed by 22 pounds. They survive one hour's steaming in the Arnold.

1. Gottheil: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1901, viii, 485.
2. Laubach: J. Bact., 1916, i, 493.

### BACILLUS FLEXUS Batchelor

Isolated from the dejecta of children by Batchelor and placed in the megatherium group because of the large size of the vegetative rods and the

early production of swollen bodies with an aggregation of the cytoplasm in globular masses. In old cultures there is a marked tendency to the development of curved or spiral forms such as are seen in *Bacillus graveolens*. The organism, however, differs in its cultural reactions from this species and from the other members of the megatherium group described in the literature.

**Morphology.**—On plain agar in young cultures the vegetative rods are large, with squared ends, measuring 1.5 to 1.8 $\mu$ in width and 2.25 to 4.5 $\mu$ in length, with many of the forms equalling 5.25 $\mu$ in length. Chain formation is frequent. The protoplasm is homogeneous in the younger elements, but rapidly becomes granular as the organisms mature. On glucose agar the larger forms predominate. On both plain agar and glucose agar the rods swell, the protoplasm segments and the elements characteristic of *Bacillus megatherium* and *Bacillus petasites* are seen in great profusion. These forms measure 1.5 to 2.2 by 4.5 to 6 $\mu$, have little of the usual cytoplasm left but in its place a mass of large highly refractile globular bodies. These are numerous in the various organisms, as many as six or eight having been observed in a single bacillus. Shadow forms develop early, often in twenty-four hours. They measure 1.2 to 1.5 by 4 to 10 $\mu$. In older cultures these shadow forms with protoplasmic remnants are very common. In such cultures long chains of organisms twisted on themselves are seen in abundance while the individual elements are often bent or curved and assume bizarre shapes. Whip forms are also common.

**Motility.**—Active progressive motility in young cultures.

**Staining Reaction.**—Gram-positive.

**Spore-formation.**—Spores are formed early on glucose agar, appearing in forty-eight hours, but are never plentiful on plain agar. They are usually terminal or subterminal and resemble megatherium spores. They are wider than the vegetative rod which bulges on sporulation. The free spores are round, measuring 1.5 by 1.5 $\mu$, or oval, measuring 1.2 by 1.5 $\mu$.

**Cultural Characters.**—*Agar Slant.*—Heavy, tallow-like growth along the line of inoculation, gradually becoming pale yellowish-brown in color.

*Agar Stab.*—Slight development along line of inoculation and tallow-like surface growth.

*Agar Plates.*—Rather profuse dirty-white, tallow-like colonies, with circumscribed edges. They vary greatly in shape, some being round and others very irregular. Under low power the colonies appear homogeneous.

*Litmus Glucose Agar.*—Rather thin growth with pellucid areas, dry, with no tendency to spread. Acidity develops in twenty-four hours and increases.

*Litmus Glucose Agar Plates.*—Thin, transparent, moist, surface colonies and punctiform deep colonies. Under low power homogeneous with regular edges. Slight acidity produced.

*Gelatin.*—Funnel-shaped liquefaction rather rapid. No scum formation.

*Gelatin Plates.*—Irregular and round dirty-white colonies, homogeneous with circumscribed edges. Rapid liquefaction.

*Broth.*—Turbidity with heavy flocculent precipitate. No scum.

*Peptone.*—Similar to growth in broth but less luxuriant. No scum.

*Potato.*—Abundant, raised, granular, cream-colored growth.

*Litmus Milk.*—Slow development of acid and later coagulation. Partial digestion of the casein.

*Blood-serum.*—Heavy, moist, yellow-brown growth. Depression of the growth with slow liquefaction of the serum.

**Fermentation Tubes.**—*Glucose.*—Turbidity and faint granular precipitate in bowl and cloudiness in closed arm. No pellicle. Reaction faintly acid.

*Sucrose.*—Turbidity in bowl and neck of tube. No pellicle. Reaction alkaline.

*Lactose.*—Turbidity in bowl and neck. No pellicle. Closed arm cloudy. Reaction acid.

**Thermal Death-point.**—Spores survive 5 pounds in the autoclave but are destroyed by 10 pounds pressure. They are killed by thirty minutes' exposure to steam in the Arnold sterilizer.

Batchelor: J. Bact., 1919, iv, 23.

### BACILLUS PSEUDOTETANICUS (Kruse) Migula

Described by Kruse[1] under the name *Bacillus pseudotetanicus aërobius*. It resembled *Bacillus tetani* in morphology and spore-formation, but was distinguished from it by its aërobic development, its failure to liquefy media and its lack of pathogenicity. Named by Migula.[2] Apparently the same organism has been described by Neide[3] as *Bacillus sphæricus*. The species

found by Ford[4] had the morphological and cultural features given by both Kruse and Neide and under the rules of nomenclature the name given by Migula should be adopted. It is apparently rare.

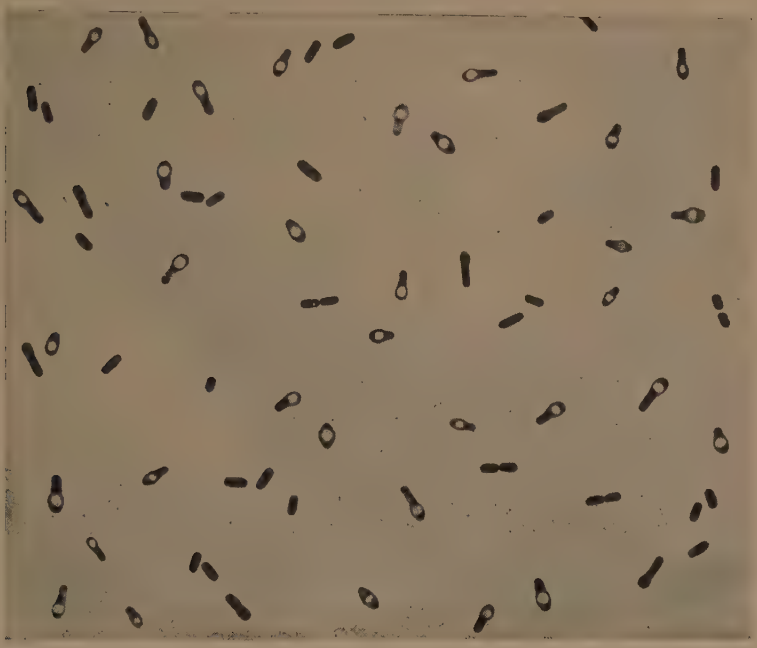


Fig. 145.—*Bacillus pseudotetanicus* (aërobic) from twenty-four-hour culture on plain agar, stained by Gram.

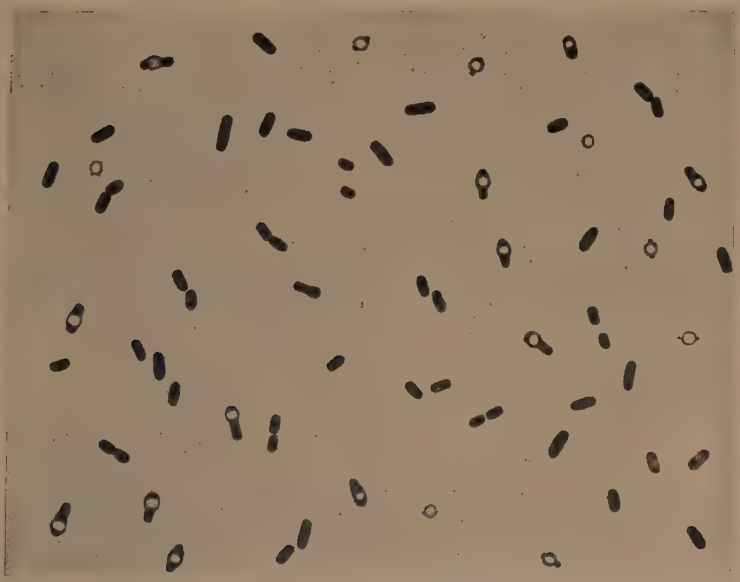


Fig. 146.—*Bacillus pseudotetanicus* (aërobic) from forty-eight-hour culture on dextrose agar, stained with gentian-violet.

**Morphology.**—Short, thick bacilli with rounded ends occurring as single cells or as two cells end-to-end. In twenty-four-hour agar cultures they measure 0.75 by 1.5 to 3 $\mu$. Protoplasm homogeneous. Many of the cells are fusiform, distinctly swollen toward the middle, while others are swollen at the ends. On glucose agar the organisms are longer and thicker, measuring 1.2 by 1.5 to 4 $\mu$. They show no change in the character of the protoplasm. In older cultures the organisms often form long threads, measuring 8 to 15 $\mu$ in length.

**Motility.**—Active and progressive rotatory motility in twenty-four-hour agar cultures.

**Staining Properties.**—Gram-negative.

**Spore-formation.**—Spores are formed early, often within twenty-four hours on both plain and glucose agar. They are round and usually appear at the ends of the organisms in a subterminal position, but may be distinctly terminal. The spores are of greater diameter than the vegetative rods from which they spring and thus give a clavate or club-like appearance to the organisms. The organisms taken from glucose agar resemble markedly the tetanus bacillus cultivated on this medium. The free spores retain fairly thick walls of protoplasm for some time. They vary in size from 1 to 1.2 $\mu$ in diameter.

**Cultural Reactions.**—*Agar Slant.*—Moist, rather thin, translucent growth in twenty-four hours, becoming pale yellowish-white in old cultures. No tendency to spread except when the medium is very moist.

*Agar Stab.*—Faint growth along the line of inoculation with slight spreading on the surface. In older cultures the surface growth is thicker and more abundant, but does not usually spread far from the line of puncture. Easily scraped from the medium.

*Agar Plates.*—Colonies round and regular or ameboid in twenty-four hours. Under low power they show dark central areas with lighter peripheries. As they age they become thicker and denser with more pronounced central nuclei.

*Glucose Agar.*—Pale white, moist, rather thin growth in twenty-four hours, becoming pale yellow or cream-yellow in older cultures.

*Glucose Agar Plates.*—In twenty-four-hour plates the colonies are irregular, ameboid, and thick, showing under low power darker central nuclei with lighter peripheries, but with entire edges. As the colonies become older they are denser and thicker with heaped-up edges. They give a peculiar bizarre appearance to the plates as though they were covered with drops of moisture. Reaction of agar remains neutral or becomes alkaline.

*Gelatin Stab.*—Faint line growth appearing after forty-eight to seventy-two hours, with a slightly spreading growth on the surface. In older cultures the surface growth spreads considerably from the point of puncture on top of the gelatin which remains solid. Rarely the line growth becomes arborescent.

*Gelatin Plates.*—Small, fine colonies visible only after forty-eight hours. Under low power they are round, regular, and pale yellow with entire margins. As they grow older they become denser and more opaque and under low power show central nuclei with pale margins. Colonies two weeks old are thick, brownish, and non-spreading. No liquefaction.

*Broth.*—Turbidity and rather faint sediment. No scum.

*Peptone.*—Turbidity with slight sediment. No scum.

*Potato.*—Rather scanty, yellowish, moist growth becoming brown in old cultures.

*Litmus Milk.*—No change in appearance of the milk in the first few days, then a gradual reduction of the litmus, completed in fifteen to eighteen days. No peptonization even in milk cultures kept under observation four weeks. No coagulation.

*Blood-serum.*—Dry, thin, yellowish growth becoming pale yellow-brown in old cultures. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Turbidity in bowl, no scum, arm clear. Reaction alkaline.

*Saccharose.*—Reaction the same.

*Lactose.*—Reaction the same.

**Thermal Death-point.**—The spores resist steaming in the Arnold sterilizer fifteen minutes but are destroyed in thirty minutes. They resist a pressure of 14 pounds in the autoclave but are destroyed by 16 pounds.

1. Kruse: *In:* Die Mikroorganismen (Flügge). 3. Aufl. 1896, ii, p. 267.
2. Migula: p. 626.
3. Neide: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1904, xii, 337; 350.
4. Ford: J. Bact., 1916, i, 493.

### Gram-positive varieties of Bacillus pseudotetanicus

The cultures of this organism described originally by the present author were uniformly Gram-negative. Recently strains have been isolated by Borges Vierra in the author's laboratory which corresponded closely in morphology and cultural reactions to the original strain, but were strongly Gram-positive.

### Bacillus fusiformis Gottheil

This organism was first reported by Gottheil.[1] A transfer from Gottheil's original was obtained from Kral's collection. Fourteen organisms corresponding closely to Gottheil's isolation were obtained in Baltimore by Ford,[2] two from milk, four from dust, two from water, five from soil, and one from contaminated hirudin. The present description applies to all of them. *Bacillus lactimorbi* of Jordan and Harris[3] has the same morphology and the same cultural reactions.

**Morphology.**—Thick stubbed homogeneous organisms with round or pointed ends, usually appearing as single cells or in twos. No chain formation. On twenty-four-hour plain agar cultures they measure 0.5 to 0.75 by 1.5 to 2 $\mu$. Organisms not increased in size on glucose agar and protoplasm remains homogeneous. Sometimes long forms, 6 to 8 $\mu$, appear in old cultures.

**Motility.**—Active, progressive, and rotary motility in twenty-four-hour agar cultures. Flagella peritrichous (Bergey).

**Staining Properties.**—Gram-negative.

**Spore-formation.**—Spores form early, appearing in twenty-four hours on both plain and glucose agar. They are round, greater in diameter than the organisms from which they spring, and are usually located at the ends of the rods in a terminal or subterminal position. They thus give a clavate or club-shaped appearance to the rods which resemble somewhat the tetanus bacilli. The spores may also be central and the rods thus become fusiform in shape. The free spores may retain spurs of protoplasm, assuming a peculiar diamond shape or may appear naked. They vary in diameter from 0.5 to 1 $\mu$, and are occasionally swollen and 1.5 $\mu$ in thickness.

**Cultural Reactions.**—*Agar Slant.*—Thick, white, rather dry growth in twenty-four hours, becoming distinctly yellow or cream-colored in old cultures. Easily scraped from medium.

*Agar Stab.*—Faint line growth and non-spreading surface growth.

*Agar Plates.*—Superficial colonies may be round, regular, thick, and opaque, or thin and spreading. Under low power they show dark central nuclei and thinner margins with clean-cut edges. Older cultures become thick and heaped-up. Deep colonies small and fine, under low power dark, opaque, round, or irregular.

*Glucose Agar.*—Thick dry growth with heaped-up edges becoming thicker and granular in old cultures. Reaction alkaline.

*Glucose Agar Plates.*—Superficial colonies thick, irregular, spreading, and heaped-up. Under low power granular with irregular fuzzy margins. Deep colonies opaque under low power, showing irregular fuzzy edges. Older colonies thicker and more bizarre-shaped. Reaction alkaline.

*Gelatin Stab.*—Growth along line of inoculation with cup-shaped or funnel-shaped liquefaction. Dense turbidity in the liquefied gelatin with a thick scum. Gelatin may be faint pink in color.

*Gelatin Plates.*—Small fine colonies, round and regular, or irregular and spreading. Under low power they show hairy outgrowths. Gelatin slowly liquefied.

*Broth.*—Turbidity and fine sediment. No scum.

*Peptone.*—Turbidity and fine sediment. No scum.

*Potato.*—Faint yellow growth becoming yellowish-brown in old cultures.

*Litmus Milk.*—Gradual reduction of the litmus and slow but complete digestion of the proteins. No coagulation.

*Blood-serum.*—Non-spreading cream-yellow growth becoming yellowish-brown in old cultures. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Turbidity in bowl. Arm clear. No scum. Reaction alkaline.

*Saccharose.*—Reaction the same.

*Lactose.*—Reaction the same.

**Thermal Death-point.**—Spores destroyed by steaming fifteen minutes in the Arnold sterilizer. They survive 7½ pounds in the autoclave, but are destroyed by 10 pounds pressure.

1. Gottheil: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1904, xii, 337.
2. Ford: J. Bact., 1916, i, 493.
3. Jordan and Harris: J. Infect. Dis., 1909, vi, 401.

### Bacillus circulans Jordan

This organism was described originally by Jordan. The present culture was obtained from normal human dejecta and corresponds closely with Jordan's description.

**Morphology.**—Rather long and thin bacilli with rounded or square ends, measuring about 0.5 by 2.5 to 4 $\mu$ in twenty-four-hour agar cultures. Occurs usually as single cells. Protoplasm homogeneous. On glucose agar the organisms are thicker and longer, measuring 0.75 by 5 to 8 $\mu$.

**Motility.**—Active, progressive, and rotatory motility in twenty-four-hour agar cultures. Flagella peritrichous (Bergey).

**Staining Properties.**—Gram-positive.

**Spore-formation.**—Spores form early on plain agar often in twenty-four hours. Abundant in forty-eight hours. Form more slowly on glucose agar. They are cylindrical, wider than the rods from which they spring, and terminal in position. The free spores may retain rims of protoplasm at one end or may be naked. They measure usually about 0.75 by 1.2 $\mu$.

**Cultural Reactions.**—*Agar Slant.*—Thin, slightly moist, non-spreading growth, pale white in color.

*Agar Plates.*—Round, regular, non-spreading colonies, under low power pale yellow, finely granular with entire edges. Older colonies become irregular and ameboid, showing under low power central nuclei and thin peripheries.

*Agar Stab.*—Faint growth along line of inoculation spreading at the point of puncture.

*Glucose Agar.*—Faint, thin, non-spreading growth producing an acid reaction beginning in twenty-four hours and quite marked at the end of five to six days.

*Glucose Agar Plates.*—Small fine colonies, under low power pale yellow and granular with entire or serrated edges. Reaction acid.

*Gelatin Stab.*—Faint growth along line of inoculation. Little or no surface growth. No liquefaction.

*Gelatin Plates.*—Small fine colonies with hazy outlines, under low power dark opaque with fuzzy margins. Older colonies quite ameboid. No liquefaction.

*Broth.*—Slight turbidity, no scum, no sediment.

*Peptone.*—Slight turbidity, no scum, no sediment.

*Potato.*—No visible growth.

*Litmus Milk.*—Complete decolorization within twenty-four to forty-eight hours. Gradual production of acidity and late coagulation which may appear spontaneously and always occurs if the milk be heated.

*Blood-serum.*—Faint, thin, white, non-spreading growth. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Turbidity in bowl and closed arm. No scum. Reaction acid, appearing in three to four days.

*Saccharose.*—Appearance the same. Reaction acid.

*Lactose.*—Appearance the same. Reaction acid.

**Thermal Death-point.**—Spores destroyed by steaming fifteen minutes in the Arnold. Survive 18 pounds pressure in the autoclave. Destroyed by 20 pounds pressure.

Jordan: Rep. Bd. Health Mass., 1890, p. 831.

### BACILLUS BREVIS Migula

This organism was first reported by Flügge[1] from milk as *Bacillus No. 1* and named by Migula.[2] According to Neide,[3] who has given an accurate account of its morphology and cultural reactions under the name *Bacillus lactic* Flügge, it is probably identical with *Bacillus cylindrosporus* Burchard. The correct name is that of Migula. Three different strains were isolated by Ford[4] from milk, from soil, and from dust. They agree in their cultural reactions with organisms studied previously in Montreal.[5]

**Morphology.**—Bacilli with pointed ends and slightly granular protoplasm, sometimes fusiform in shape. In twenty-four-hour cultures on plain agar they measure 0.4 to 0.5 by 2 to 3 $\mu$. On glucose agar they are more uniform and slightly thicker and longer, measuring usually 0.5 by 3 to 4 $\mu$.

**Motility.**—Active, progressive motility in twenty-four-hour agar cultures. Flagella peritrichous (Bergey).

**Staining Properties.**—Gram-negative.

**Spore-formation.**—Spores are formed early, appearing often within twenty-four hours on plain and glucose agar. They are cylindrical, usually subterminal in position, thicker than the organisms from which they spring, and present a characteristic appearance. They may retain spurs of protoplasm at each end or may be entirely naked. They measure usually 0.75 by 1.125 to 1.5 $\mu$. Rarely they may be distinctly terminal in position.

**Cultural Reactions.**—*Agar Slant.*—Thick, moist, spreading growth with a silvery sheen, abundant in twenty-four hours, and becoming thick white and glistening in older cultures. Easily scraped from medium.

*Agar Stab.*—Filiform growth along the line of inoculation with an abundant surface growth spreading to the tube wall.

*Agar Plates.*—Surface colonies in twenty-four hours are round and regular or thin, spreading, and translucent, under low power slightly granular with entire edges. Deep colonies small and fine, under low power irregular and pale yellow. As the superficial colonies get older they become thicker and heaped-up slightly. The deep colonies may show irregular fuzzy margins under the low power.

*Glucose Agar.*—Heaped-up, white, moist, non-spreading growth in twenty-four hours, becoming thick and raised in old cultures. Reaction alkaline.

*Glucose Agar Plates.*—Superficial colonies are slightly thicker than plain agar colonies in twenty-four hours and show a greater tendency to spread. Under the low power they are granular with entire edges. Deep colonies small and fine, under low power irregular and pale yellow. Older superficial colonies are thick, irregular, and heaped-up. Reaction of agar alkaline.

*Gelatin Stab.*—Faint growth along line of inoculation and at the point of puncture in three to four days, with beginning liquefaction. Distinct cup-shaped or funnel liquefaction in two weeks. Occasionally a fragile scum appears on the liquid gelatin.

*Gelatin Plates.*—Colonies begin to appear in forty-eight hours and show dark central areas with hazy peripheries. Under the low power they show dark opaque centers with wavy branching peripheries. Deep colonies frequently show peculiar prickly margins. Gelatin completely liquefied at the end of about two weeks and may show a thick scum on the surface.

*Broth.*—Turbidity and a flocculent sediment in twenty-four to forty-eight hours, with a gradual production of a heavy scum which appears often only after four days. In old cultures the precipitate is quite heavy.

*Peptone.*—Turbidity and heavy flocculent sediment. Scum may be thick and heavy or entirely lacking.

*Potato.*—Usually a rather scant, moist, yellow, or yellow-brown growth.

*Litmus Milk.*—No change in reaction. Litmus begins to decolorize in forty-eight hours and by the end of two weeks is completely decolorized. At the same time the proteins are digested and the resulting fluid is a pale, rather thin, colorless solution. No coagulation. According to Lisk[6] *Bacillus brevis* shows an increase of ammonia and aminonitrogen in milk, some acetic acid, and marked indol production. No hydrogen sulphid or mercaptan.

*Blood-serum.*—Thin, cream-white, non-spreading growth becoming yellowish-brown in old cultures. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Thick scum, turbidity in bowl, arm clear. Reaction alkaline.

*Saccharose.*—Reaction the same.

*Lactose.*—Reaction the same.

**Thermal Death-point.**—The spores survive steaming thirty minutes, but are destroyed by forty-five minutes exposure. They may survive a pressure of 16 pounds in the autoclave, but are destroyed by 18 pounds pressure.

1. Flügge: Ztschr. f. Hyg., 1894, xvii, 294.
2. Migula: p. 583.
3. Neide: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1904, xii, 1.
4. Ford: J. Bact., 1916, i, 493.
5. Ford: Studies Royal Victoria Hospital, 1901–03, i, No. 5.
6. Lisk: J. Bact., 1924, ix, 1.

### BACILLUS TERMINALIS Migula

This organism was first obtained by Flügge[1] and called by him *Bacillus* No. XII. It was subsequently correctly named *Bacillus terminalis* by Migula.[2] On two occasions Lawrence and Ford[3] isolated organisms which had the same morphology and method of spore-formation as *Bacillus terminalis*, but differed slightly in cultural reactions. It does not seem wise to make a new species since these strains may represent merely attenuated varieties of Flügge's organism. The following description is taken from the strains of Lawrence and Ford and points of differentiation between them and Flügge's original are indicated.

**Morphology.**—Long, thin bacilli with slightly granular protoplasm measuring 0.4 by 2.25 to 4 $\mu$ in twenty-four-hour agar cultures. On glucose agar the organisms retain the same diameter, but grow out into long chains which often assume spiral arrangements.

**Spore-formation.**—Spores are formed slowly, seldom appearing before forty-eight hours. They are cylindrical, thicker than the rods from which they spring, terminal, or subterminal, giving the organisms a clavate or club-shaped appearance. Free spores are 0.75 by 1.5 $\mu$ in dimensions.

**Motility.**—Active motility in twenty-four-hour culture. Flagella peritrichous (Bergey).

**Staining Properties.**—Gram-negative.

**Cultural Reactions.**—*Agar Slant.*—Thin, spreading, smooth, glistening growth with gradual darkening of the agar.

*Agar Stab.*—Faint growth along the line of puncture and on the surface at the point of inoculation.

*Agar Plates.*—Colonies grow slowly appearing only after three to four days. They are round and regular, under low power showing central nuclei with thin spreading peripheries. Deep colonies apt to be irregular under low power, showing clean cut or entire edges.

*Glucose Agar.*—Faint, white, filmy growth with an alkaline reaction.

*Glucose Agar Plates.*—Thin, slow-growing, spreading, surface colonies, under low power showing dense central nuclei and thin margins. Deep colonies punctiform, under low power slightly granular with irregular margins. Reaction alkaline.

*Gelatin Stab.*—Growth along line of inoculation and slow cup-shaped liquefaction.

*Gelatin Plates.*—Colonies on the surface show dense central nuclei and concentric spreading peripheral margins. Deep colonies punctiform and tend to show a similar arrangement. Under low power edges entire.

*Broth.*—Slight turbidity. No scum. No sediment. Fragile scum described by Flügge.

*Peptone.*—Slight turbidity. No scum. No sediment.

*Potato.*—No visible growth in the isolations of Lawrence and Ford. Faint moist growth gradually becoming thicker and yellowish, noted by Flügge.

*Litmus Milk.*—No change produced by the strains of Lawrence and Ford. Slow peptonization described by Flügge.

*Blood-serum.*—Thin, transparent, spreading growth, pale yellow to yellowish brown. No liquefaction. Slight sinking-in of the growth mentioned by Flügge.

**Fermentation Tubes.**—*Glucose.*—Faint turbidity in bowl. No scum. No growth in closed arm. Reaction alkaline.

*Saccharose and Lactose.*—Appearance the same. Reaction alkaline.

**Thermal Death-point.**—Spores survived 10 pounds in the autoclave but were killed by 15 pounds pressure. Flügge's organisms were not killed by boiling five hours.

1. Flügge: Ztschr. f. Hyg., 1894, xvii, 296.
2. Migula: p. 578.
3. Lawrence and Ford: J. Bact., 1916, i, 273.

### Bacillus centrosporus Ford

Obtained by Ford from the normal dejecta of a child.

**Morphology.**—In twenty-four-hour cultures on plain agar at 37° C. or in thirty-six-hour cultures at 22° C. long, thin bacilli with pointed ends, fusiform in shape with slightly granular protoplasm, occurring usually as single cells measuring 0.4 to 0.5 by 3 to 4.5 $\mu$ in dimensions. The majority of the organisms, measure about 3 to 4 $\mu$ in length. On glucose agar the organisms measure about 0.4 by 2.25 to 3 $\mu$ and the ends are rounder but there is no change in the character of the cytoplasm.

**Motility.**—Active, progressive and rotatory motility in twenty-four-hour agar culture.

**Staining Properties.**—Organisms stain rapidly by the ordinary dyes. Gram-negative.

**Spore-formation.**—Spores are formed early, appearing in twenty four hours on plain agar in the thermostat, somewhat later on glucose agar. They are usually formed in the central portion of the rods, causing a distinct swelling so that the bacilli become spindle-shaped. Rarely the spores appear toward the end of the rods in a subterminal position or they may even be terminal. They are much wider than the vegetative rods and when free are distinctly cylindrical with pointed spurs. They measure 0.6 by 1.2 to 1.5 $\mu$. The spores are abundant by the end of forty-eight hours, both at 37° C. and at 22° C.

**Cultural Reactions.**—*Agar Slant.*—Thick, moist, rather dark, non-spreading growth in twenty-four hours, becoming thicker and raised in forty-eight hours and in older cultures. Easily scraped off from medium.

*Agar Stab.*—Growth along the line of inoculation in twenty-four hours, gradually spreading on the surface of the agar at the point of puncture.

*Agar Plates.*—Round, regular, rather translucent colonies in twenty-four hours, granular with smooth margins under the low power. Older colonies show pale central areas with heaped-up peripheries, but remain small, regular, and slightly raised, resembling those of *Bacillus coli.*

*Glucose Agar Slant.*—Thick, moist, cream-yellow, non-spreading growth in twenty-four

hours, becoming thick and heavy with an irregular surface of a reddish-brown color in old cultures. Reaction alkaline.

*Glucose Agar Plates.*—Irregular heaped-up colonies in twenty-four hours, tending to become slimy or viscid-looking. Under low power they are irregular, heaped up or swollen, with entire margins. At times they have a dew-drop appearance. On old cultures (ten days) they are thicker and slightly brownish. Reaction of medium alkaline.

*Gelatin Stab.*—Filiform growth along the line of inoculation and gradual liquefaction in the form of a sphere below the surface. Liquefaction is slow, taking place only after seven to eight days. Liquefaction gradually reaches the walls of the tube. Growth not arborescent.

*Gelatin Plates.*—Colonies appear only after five to six days. They are small, fine, and surrounded by a hazy zone. Gelatin slowly liquefied. Under low power colonies are round, regular, finely granular with entire margins or may show irregular outlying strands.

*Broth.*—Turbidity within twenty-four to forty-eight hours with a faint friable scum appearing in about ten days.

*Peptone.*—Turbidity within twenty-four to forty-eight hours with faint friable scum in ten days.

*Potato.*—Moist, yellow growth within twenty-four hours gradually becoming heaped-up and irregular and assuming a brown or reddish-brown color.

*Litmus Milk.*—No change in reaction except for an occasional partial reduction of the litmus. No peptonization. No coagulation.

*Blood-serum.*—Pale yellow growth in twenty-four hours gradually becoming thick and moist in old cultures. No liquefaction.

**Fermentation Tubes.**—*Glucose.*—Turbidity in bowl. No scum. Arm clear. Reaction alkaline.

*Saccharose.*—Reaction the same.

*Lactose.*—Reaction the same.

**Thermal Death-point.**—The spores stand boiling fifteen minutes, but are destroyed by boiling for thirty minutes. They resist fifteen minutes' exposure in the Arnold, but are destroyed by thirty minutes' exposure. They resist autoclaving at a pressure of 12½ pounds but are destroyed by 14 pounds pressure.

Ford: J. Bact., 1916, i, 493.

### BACILLUS LATEROSPORUS Laubach

Described by Laubach from water.

**Morphology.**—The typical vegetative rods are found only in very young cultures six to eight hours old. They are homogeneous or slightly granular with round ends, measuring 0.4 to 0.5 by 1.2 to 4 $\mu$ on plain agar. On glucose agar they are slightly thicker and about the same length, measuring 0.5 to 0.75 by 1.2 to 4 $\mu$. The rods rapidly lose their characteristic appearance and assume a fusiform or spindle shape with a swollen middle and pointed ends. Such forms take the stain much less intensely.

**Motility.**—Active motility in young cultures.

**Staining Properties.**—Gram-positive.

**Spore-formation.**—As the organisms swell and assume the fusiform shape the spores appear at one side as slightly staining globules. The protoplasm soon disintegrates but more on one side of the spore than on the other so that the free spores, which are almost egg-shaped, retain a rim of protoplasm on one side and present a characteristic appearance. Such spores measure 0.75 to 0.9 by 1.5 $\mu$. As the spores mature they lose their rims of protoplasm, become more oval and measure 0.75 to 1 by 1 to 1.2 $\mu$. The spores begin to appear in about forty-eight hours and are abundant in seventy-two hours. Rarely small tags of protoplasm remain attached at the ends of the spores.

**Cultural Characters.**—*Agar Slant.*—The growth is very characteristic. It is profuse, rapidly spreading, smooth, moist, and has a silvery metallic-like lustre. Within seventy-two hours the growth becomes slightly wrinkled. With age the growth loses its metallic lustre, becomes dull and scrapes off in scales.

*Agar Stab.*—A slight grayish, granular growth along the line of stab.

*Agar Plates.*—The colonies are flat, transparent, irregular, moist, and have the same peculiar metallic lustre.

*Glucose Litmus Agar Slant.*—Growth slight, granular, moist, and gray. Slight reduction of the litmus within twenty-four hours and a permanent acidity within ninety-six hours.

*Glucose Litmus Agar Plates.*—Colony formation is very slow and the colonies only appear within forty-eight hours. They are similar to those on agar but less profuse. At times a slight wrinkling occurs.

*Gelatin Stab.*—Very slow surface liquefaction.

*Gelatin Plates.*—The colonies are definitely circumscribed, gray, and moist, under the low power of the microscope showing finely granular centers surrounded by small, gray rings.

Fig. 147.—*Bacillus laterosporus* from twenty-four-hour culture on plain agar, stained with gentian-violet.

Fig. 148.—*Bacillus laterosporus* from three-day culture on blood-serum, stained with gentian-violet.

Beyond the rings there are other granular areas, the outer borders of which consist of dense gray rings. A very peculiar odor like that of semen plus some aromatic radical occurs in gelatin.

*Broth.*—Peculiar pellicle made up of discrete colonies which sink to the bottom. There is also a slight turbidity.

*Potato.*—The growth is dull, moist, gray, granular, and finely wrinkled. A slight lustre appears which becomes more pronounced as the medium becomes drier.

*Litmus Milk.*—Within twenty-four hours there is a complete reduction of the litmus with an alkaline ring at the surface. A soft coagulum is formed within twenty-four hours, which is somewhat firmer than the coagulum formed by other spore-bearing organisms. Peptonization begins after forty-eight hours and requires a considerable period of time before it is completed. The remaining fluid is straw-colored.

*Blood-serum.*—The growth is grayish, moist, non-spreading, and not profuse. Within ninety-six hours it becomes finely wrinkled.

**Fermentation Tubes.**—*Glucose.*—There is a heavy pellicle formed and a turbid growth in the bowl and neck of the tube. If litmus is present it is reduced in the closed arm. Also a turbid growth is found in this arm. Reaction acid.

*Saccharose.*—A similar condition is apparent with little or no acidity.

*Lactose.*—There is the same reaction with a slight alkalinity.

**Thermal Death-point.**—The spores survive 12 pounds pressure in the autoclave, but are destroyed by 15 pounds pressure. They are destroyed by one hour's steaming in the Arnold sterilizer.

Laubach: J. Bact., 1916, i, 493.

### BACILLUS FUSUS Batchelor

Isolated by Batchelor from the dejecta of children. Morphologically it is similar to *Bacillus centrosporus* of Ford. It is differentiated by its staining reactions and by its cultural features.

**Morphology.**—In twenty-four-hour cultures on plain agar the vegetative rods lie singly, are thin and delicate with homogeneous protoplasm. They vary greatly in size and shape because of the early sporulation. They are usually regular in outline with round ends and measure 2.25 to 4.5 $\mu$ in length by 0.75 $\mu$ in thickness. The longer forms predominate. On glucose agar the rods are thicker, measuring 1.2 $\mu$ and show many long forms, 4 to 6 $\mu$, with whip forms 12 to 20 $\mu$ in length. In older glucose agar cultures the whip forms are much longer, often running across the field of the microscope or lying wound up in long coils. They are usually thinner than the short rods, having an average width of about 0.75 $\mu$. In cultures four to five days old involution forms begin to appear, small heavily staining globular masses measuring 1.5 to 1.9 $\mu$ in width by 2.25 to 3.75 $\mu$ in length. Shadow forms are common on plain agar.

**Motility.**—Active progressive motility in young broth cultures.

**Staining Reactions.**—Gram-positive.

**Spore-formation.**—Sporulation begins on plain agar in twenty-four hours but seldom occurs in glucose agar where the involution forms predominate. The spores form in the center of the rods which become pointed as the spore develops. The rods thus take on a spindle-shape. The sporangia are often irregularly swollen with one or both ends pointed. As the spores mature the organisms are bulged, becoming very thick centrally because of the size of the spore. The free spores are large compared with the rods, oval, and measure 1.2 by 2.25 $\mu$. They do not retain protoplasmic tags but are provided with a rather thick, outer rim of ectoplasm (exine). Great variation is seen in the size and shape of the spores. Some remain oval, others are longer, measuring 1.2 by 3 $\mu$. Others are swollen, measuring 1.9 by 2.25 $\mu$. At times the sides are flattened and the spores become reniform.

**Cultural Characters.**—*Agar Slant.*—Thin, white, beaded growth never becoming very abundant. Growth at times adherent to the medium.

*Agar Stab.*—Faint growth along line of inoculation with a slight bead-like knob at the point of puncture.

*Agar Plates.*—Small, pin-point, white colonies, smooth and round, with dark centers and pale peripheries. Under low magnification, homogeneous with complete edges.

*Litmus Glucose Agar.*—Growth more abundant than on plain agar, beaded, without tendency to spread. Easily scraped from the medium. Gradually increasing and persistent acidity.

*Litmus Glucose Agar Plates.*—Small, round, white colonies, larger than on plain agar. Under low power usually homogeneous with complete edges, but the colonies may show fine reticulation.

*Gelatin.*—Faint growth along line of inoculation. No liquefaction.

*Gelatin Plates.*—Small, irregular, or round, pale colonies with well-defined edges. No liquefaction.

*Broth.*—Slight turbidity and granular precipitate. No scum.

*Peptone.*—Slight turbidity and granular precipitate. No scum.

*Potato.*—Moist, creamy, rather abundant growth, at times pale brown in color.

*Litmus Milk.*—Gradual reduction of the litmus without digestion of the proteins or coagulation.

*Blood-serum.*—Very faint, dry, and hardly visible growth.

**Fermentation Tubes.**—*Glucose.*—Faint turbidity with a slight granular precipitate in the bowl and neck. No scum. Reaction acid.

*Sucrose.*—Faint turbidity, slight precipitate. No scum. Reaction acid.

*Lactose.*—Faint turbidity with fine granular precipitate. No scum. Reaction acid.

**Thermal Death-point.**—Spores survive 5 pounds pressure in the autoclave, but are destroyed by 7 pounds. They resist steaming in the Arnold for fifteen minutes, but succumb to thirty minutes' exposure.

Batchelor: J. Bact., 1919, iv, 23.

### BACILLUS ASTEROSPORUS Meyer

Cultivated originally by Meyer[1] from decaying carrots and characterized by its ability to produce gas from carbohydrates. Widely distributed in soil, decaying vegetables, and occasionally present in water where it interferes with the presumptive test for *Bacillus coli.* Isolated from milk by Lisk,[2] whose description follows.

**Morphology.**—Vegetative rods on plain agar in twenty-four hours, of medium length and rather slender. They vary in width from 0.6 to 0.7 $\mu$ and in length from 2 to 3.5 $\mu$. They are frequently curved, slightly larger at one end and may be fusiform. Darkly staining granules are occasionally present in the centers of the rods. No capsules.

**Motility.**—Active. Flagella peritrichic, about ten in number, easily demonstrated by Loeffler's method.

**Stained** easily by the usual anilin dyes. Gram-positive in twenty-four-hour cultures.

**Spores** begin to form in twenty-four hours and are abundant in forty-eight hours. They are central, excentric or terminal, much wider than the vegetative rods. Free spores oval, regular in outline, about 1.4 $\mu$ in width by 2.1 $\mu$ in length.

**Cultivated** on ordinary media under both aërobic and anaërobic conditions.

**Cultural Characters.**—*Plain Agar Plates.*—Colonies very small, irregular or round, translucent, thin, white, adherent to the agar. They may be $\frac{1}{8}$ inch in diameter but are usually smaller. Occasionally delicate, filmy, fern-like colonies are present, $\frac{3}{8}$ inch in diameter.

*Plain Agar Slant.*—Thin, beaded, translucent growth in twenty-four hours, adherent to the agar. In forty-eight hours growth usually spreads over the medium at the base of the slant and presents an irregularly serrate outline.

*Plain Agar Stab.*—Thin, blade-like growth along the line of the stab, with crenate margins. Delicate, beaded, translucent surface growth at point of inoculation. In old cultures the line growth shows fine, bristle-like projections. Gas bubbles appear in the depths of the medium.

*Dextrose Litmus Agar Slant.*—Slight dotted growth in twenty-four hours. Medium acid, becoming alkaline in six to seven days. Gas produced in the medium.

*Gelatin Stab.*—Faint, delicate line growth in twenty-four hours. Liquefaction develops about the fourth day as a large bubble-like depression immediately below the surface, filled with flocculent growth. Definite liquefaction appears in this area after twelve to thirteen days.

*Endo's Medium.*—Small, round colonies varying from a pin-point to $\frac{1}{8}$ inch in diameter. Colonies reddened but show no sheen. Old colonies tough, raised, with marked central depressions. Gas bubbles appear in the medium.

*Blood-serum.*—Dotted line growth in twenty-four hours. The medium becomes translucent beneath the surface but is not liquefied.

*Potato.*—Milky-white growth in twenty-four to forty-eight hours, coarsely punctate, or so foamy as to be blown against the sides of the tube. Gas production up to 20 per cent. in twenty-four hours and up to 50 per cent. in forty-eight hours. The growth turns yellow after four to five days and gradually becomes dark brown.

*Litmus Milk.*—Acidity in twenty-four hours, soft coagulation in forty-eight hours with a reduction of the litmus. Digestion of the clot takes place fairly early and by the fourth day the digested zone is amber and the coagulum a pale tan. The coagulum has a rubber-like consistency and is shot with gas bubbles. After twelve to fourteen days the digested zone is bright red in color and remains so indefinitely. A portion of the coagulum remains undigested on the thirtieth day.

*Broth.*—No scum. Faint, dotted ring growth along the sides of the tube, slight turbidity, and sediment in the depths.

*Peptone.*—No scum. Faint, dotted ring growth, slight turbidity, and sediment.

Methyl red reaction negative. Voges-Proskauer positive. No indol. Nitrates reduced to nitrites.

**Fermentation Tubes.**—*Dextrose.*—Scum formed in twenty-four to forty-eight hours, marked turbidity and slimy growth. Gas produced in abundance, 60 to 100 per cent. in seventy-two hours, $\frac{H}{CO_2} = \frac{2}{1}$.

Fermentation also of lactose, saccharose, maltose, levulose, inulin, and mannite.

*Russell Andrade Medium.*—Acidity in dextrose and lactose, reverting to neutral in five to six days. Gas produced in the medium.

1. Meyer: Sitzungsb. d. Gesellsch. z. Beförd. d. ges. Naturw. zu. Marb., 1897, p. 49.
2. Lisk: J. Am. Water Works Ass., 1923, x, 139.

### Bacillus cyanogenus Flügge

Found in blue milk by Ehrenberg.[1] Known also as *Bacterium syncyaniun*, *Bacillus lactis cyanogenus*, and the "bacillus of blue milk." Description from Flügge.[2]

**Morphology.**—Bacilli with slightly rounded ends from 0.3 to 0.5 $\mu$ broad and from 1.4 to 4 $\mu$ long.

**Motility.**—Active.

**Spore-formation.**—Spores at the ends of the rods, giving them a club-shape.

**Cultural Characters.**—Grows rapidly at 22° C. and at 37° C.

*Slant Agar.*—Grayish expansion, the agar acquiring a dark brown color near the surface.

*Gelatin Plates.*—Small punctiform grayish-white deep colonies in forty-eight hours. Superficial colonies like slimy drops which attain a diameter of 1 to 2 mm. They are finely granular, dirty-white in color, and have circular, smooth outlines. The gelatin about them acquires a steely, grayish-blue shimmer. Under low power deep colonies are round disks with dark centers and brownish, granular, marginal zones with sharply defined, dark contours. Superficial colonies under low power have dark brown centers surrounded by grayish-brown zones outside of which are narrow, yellowish, finely granular, marginal zones with sharply defined contours.

*Gelatin Stab.*—Growth chiefly at the point of puncture as a white mass about which the gelatin acquires a dark steel-blue color.

*Potato.*—Slimy yellow growth along line of inoculation about which the potato acquires a diffused grayish-blue color.

*Blood-serum.*—Non-pigmented growth.

*Milk.*—Slight alkaline reaction. No coagulation. Slate-blue pigment at the surface and gradually extending downward. This changes to an intense blue upon the addition of an acid. In milk containing acid-producing bacteria and not sterilized, the organism produces a sky-blue color.

Pigment slate-blue to sky-blue, produced most abundantly at 15° to 18° C. Less abundant at 25° C., not formed at 37° C.

1. Ehrenberg: Ber. u. die Verhandl. d. Berl. Akademie, 1840, p. 202.
2. Flügge: Die Mikroorganismen. 3. Aufl. 1896, ii, 294.

### Bacillus anthracis Koch

Rod-shaped elements were seen in the blood, in anthrax, splenic fever, or "charbon" by Pollender[1] in 1849 and reported in 1855. Found also by Brauell[2] in 1857–58 in the blood of man, sheep, and horses dead of anthrax. Brauell succeeded in transferring the disease from animal to animal. Davaine and Rayer in 1850 called attention to peculiar structures in the blood of animals with anthrax. Established as cause of disease by Davaine in 1863, only blood containing the peculiar rods being infectious. The question was cleared up by Koch[3] in 1876, who showed that the organisms belonged to the group of "bacilli" of Cohn, cultivated them outside the body, and produced the disease by pure cultures. Koch described the spores and worked out the complete life cycle.

**Morphology.**—In the blood of infected animals the organisms appear as thick non-motile rods, measuring 1 to 1.25 $\mu$ in thickness by 4 to 10 $\mu$ in length, and occurring as single cells or short chains of two or three individuals. Organisms from plain agar seven hours old measure

Fig. 149.—*Bacillus anthracis* from seven-hour culture on plain agar, stained with gentian-violet.

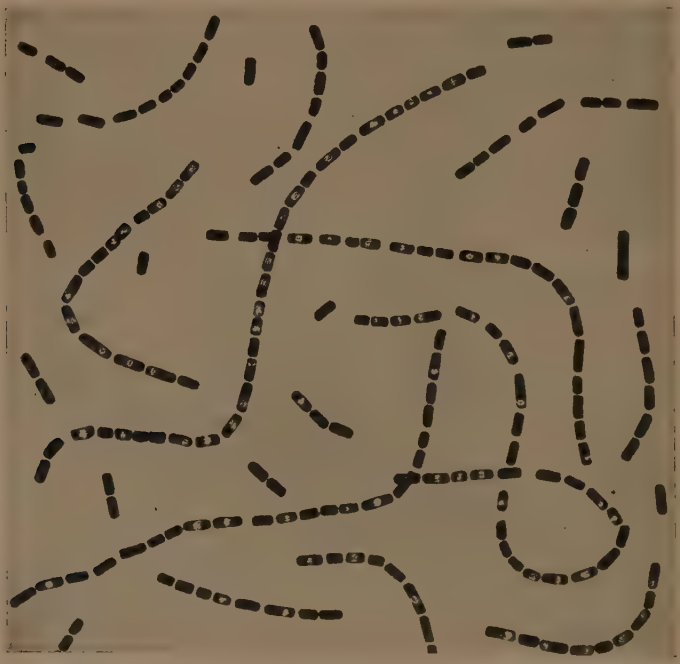


Fig. 150.—*Bacillus anthracis* from plain agar, twenty-four hours old, stained with gentian-violet.

Fig. 151.—*Bacillus anthracis* from dextrose agar, twenty-four hours old, stained with gentian-violet.

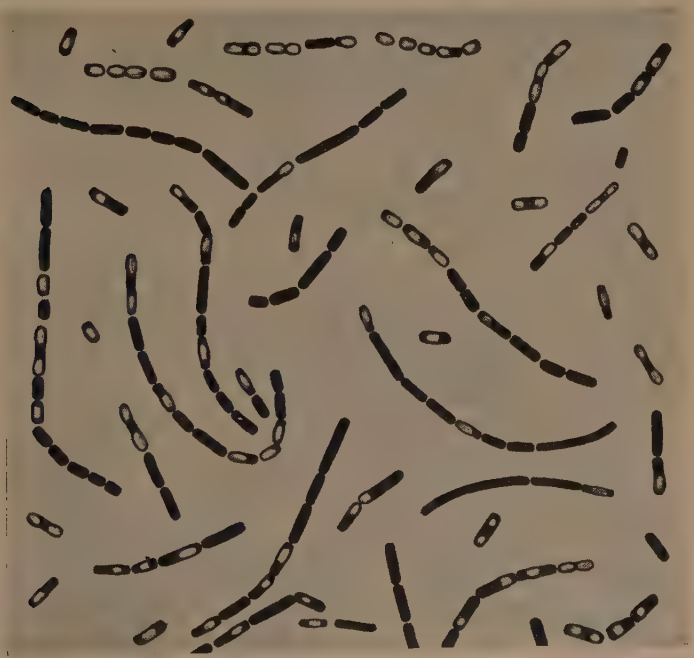


Fig. 152.—*Bacillus anthracis* from four-day culture on plain agar, stained by Stirling Gram.

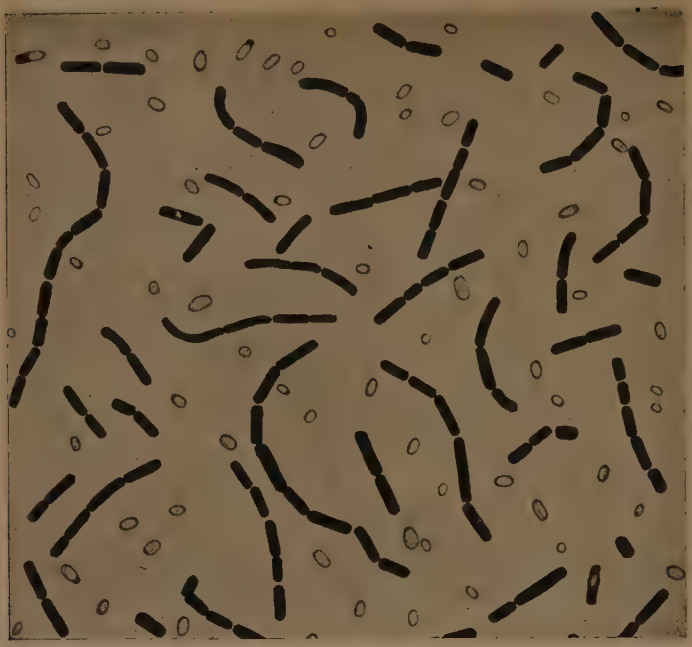


Fig. 153.—*Bacillus anthracis* from ten-day culture on plain agar, stained by Stirling Gram.

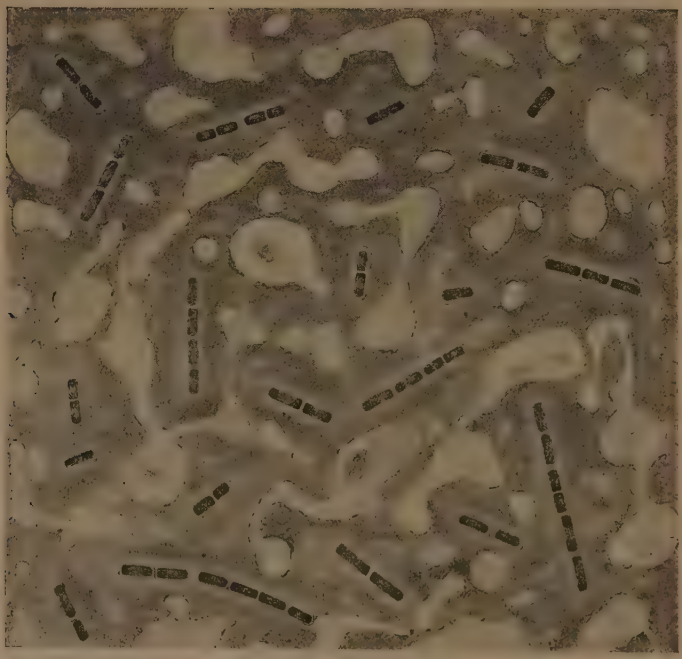


Fig. 154.—*Bacillus anthracis* from heart blood of guinea-pig, stained by Hiss's capsule stain.

usually 0.75 to 0.9 by 3.5 to 4.5 μ. Some organisms are shorter, measuring 0.75 by 2.8 μ. Ends square. Many chains occur containing four to twenty elements. In twenty-four hours on plain agar the organisms when stained by gentian-violet appear somewhat shorter, measuring 0.75 by 2 μ, each organism now showing a central spore. There are also tangled threads and chains in skeins. On dextrose agar seven hours old the organisms are somewhat larger, measuring 1 by 3.5 to 4 μ. Cultures on dextrose agar twenty-four hours old show chiefly swollen organisms, measuring 1.5 by 2.5 μ or 1.5 by 3 μ, and containing globular bodies about 0.75 by 1 μ in diameter. In older cultures in broth, plain and dextrose agar there is great diversity of form, swollen rods, long threads, short and long chains of short forms appearing, together with rods containing spores, many free spores and many spores in chains. Klett[4] says that the individual rods are made up of coherent homogeneous material, but have several elements arranged in different layers. These may be seen in dried unstained preparations. They consist of an outer capsule (Plasmahülle), a segmented plasma body, and a nuclear rod (Schottelius[5]).

**Staining Reactions.**—*Bacillus anthracis* stains well by carbolfuchsin, gentian-violet, methylene-blue, and retains Gram's stain, appearing dense blue-black. When stained the individual rods may show clubbed extremities or the ends may seem to be slightly convex. Such organisms in chains look like bamboo rods and are characteristic, but not universal.

**Capsules.**—In blood and animal tissues the organisms are encapsulated. The capsules may be demonstrated by various methods, those of Friedländer, Johne, Klitt, Olt, and Räbiger, giving excellent results. By staining in Ziehl's solution two hours at 60° to 70° C., treating with aqueous solution of potassium bicarbonate (1 : 10,000) and counterstaining with methylene-blue, the bacilli are stained blue, the capsules red. By staining with alcoholic aqueous methylene-blue (1 : 10 : 100) and counterstaining with fuchsin of the same concentration (Klett) the bacilli appear blue, the capsules rose-colored. When stained by Romanowsky's eosin methylene-blue the bacilli appear to be made up of red-stained chromatin masses and blue plasma substance enclosed in a bright red capsule. Capsules may also be demonstrated by the methods of Hiss, Welch, Wadsworth, and Huntoon. Capsules do not ordinarily appear in cultures. They may be demonstrated, however, in cultures in fluid blood-serum (Johne), on agar containing hen protein (Kodama), and in blood-serum containing glucose, levulose, saccharose, maltose, and glycogen.

**Involution Forms.**—In certain media the anthrax bacillus shows great variation from the typical morphology. Thus in fluid media like the aqueous humor of the eye threads of various lengths develop in a few hours. They are often very long, films showing them running through the entire field of the microscope. By the end of ten to twelve hours tangled masses are seen resembling bunches of hair or coils of a ship's hawser. Later these long threads produce spores, and long chains of oval spores may be found. Other types of involution forms may also be seen, swollen sausage-shaped thickened elements, spirals, large spore-like bodies, etc. Such forms have sometimes been described as distinct species, *Bacillus anthracis claviformis* (Chauveau) or *Bacillus anthracis brevigemmans* (Phisalix).

**Spore-formation.**—*Bacillus anthracis* produces spores abundantly in the presence of atmospheric oxygen, but not in the blood or the body of animals dead of anthrax, oxygen being necessary for their formation. They form rapidly at high temperatures, in sixteen hours at 31° to 33° C., in thirty-six hours at 24° C., in fifty hours at 18° C. The optimum temperature for spore-formation lies between 25° and 35° C. In the process of spore-formation the bacterial substance becomes less opaque and appears powdery with fine granules. Then highly refractile granules appear, sometimes called sporogenes granules (Bunge), which may be stained by hot methylene-blue. These refractile granules lie in the middle of each rod, but as they develop into spores they pass to one or the other end, producing a sporangium, and the bacterial cell degenerates and disappears. Frequently the spore carries a tag of protoplasm for some time. On plain and dextrose agar spores are formed abundantly by the end of forty hours. They usually measure 0.5 by 0.75 μ or 0.75 by 1 μ. Older cultures, three to five days, show a great mass of spores, often in chains. Some are much larger, measuring 0.7 by 1.4 μ. In cultures eight to ten days old the organisms are almost entirely converted to spores, only a few distorted bacilli being present. Mature spores are highly refractile and egg-shaped. Nakanishi[6] believes that they are made up of a hard membrane, an inner endosporium, and an outer delicate ectosporium at the poles, with a half-moon intermediate space between. The spores may be stained by hot anilin water fuchsin or carbolfuchsin applied for two to three minutes, decolorizing in alcohol and then counterstaining with methylene-blue (Moeller's method). According to Dyrmont spores have more protein material than vegetative rods, 77.75 per cent. for the spores as compared with 42.5 per cent. for the rods.

**Spore Germination.**—Under proper conditions the spores germinate and produce vegetative rods. They first enlarge, become drawn out and egg-shaped, then lose refractility, disintegrate, and disappear, leaving the vegetative rod. Prazmowski[7] has observed the vegetative rod emerges from a polar hole at one end of the long axis, shedding the spore membrane which then lies behind the bacillus. Nakanishi found both polar and equatorial germination in *Bacillus anthracis*.

**Asporogenous strains** of *Bacillus anthracis* have been observed by Lehmann[8] and have been obtained by Behring[9] by cultivation in gelatin containing minute traces of bactericidal substances, HCl, sodium hydrate, rosolic acid, litmus tincture, safranin, and malachite green. They have likewise been obtained by Roux[10] by cultivation in phenol-broth, no spores appearing in a concentration of 20 : 10,000. After eight to ten days transfers gave asporogenous strains which remained so without loss of virulence. Eisenberg[11] believes that there are two races or strains of *Bacillus anthracis*, often together in laboratory cultures, one sporulating, one non-sporulating. By heating to 70° to 90° C. the sporogenous strain may be obtained by frequent transfer of young cultures, the asporogenous strain. The sporogenous strain may be converted into the asporogenous by cultivation on glycerin agar or less constantly on glucose agar, after five to twenty passages. The asporogenous strains generally have lessened virulence.

**Cultivation.**—*Bacillus anthracis* may be cultivated without difficulty on all the usual laboratory media. The growth is abundant, spreading, and characteristic. Growth takes place at temperatures ranging from 15° to 43° C. Below 15° C. there is usually no growth, but according to Diedonné the organism can be accustomed to a temperature of 12° to 16° C. It grows but sparsely under anaërobic conditions.

**Cultural Characters.**—*Plain Agar Plates.*—Surface colonies grow rapidly, appearing in twenty-four hours as dense, opaque, irregular masses, about ½ to 2 mm. in size. Under low power they show tangled skeins of interlacing threads, irregular margins, and branching or irregular radiating outgrowths. Deep colonies small, usually round or oval. Under low power they are much like the surface colonies, but denser and more compact. The colonies increase rapidly in size, those on the surface becoming very irregular in shape. After three to four days the latter measure up to ½ cm. By the end of ten days they are much larger, thick, elevated, and irregular, up to 1 cm. in diameter. Bottom colonies thin and spreading with irregular margins.

*Plain Agar Slant.*—Thick, dull white, abundant raised growth in twenty-four hours. It increases rapidly in breadth and thickness and later becomes granular. By the end of ten days a thick heavy spreading expansion with irregular margins is formed.

*Plain Agar Stab.*—Abundant growth along the line of inoculation in twenty-four hours with a surface expansion spreading from the point of puncture. By the end of the second day the line growth sends out irregular radiations and the whole mass suggests the appearance of an inverted fir tree. In three to four days the line and surface growth are much more marked and the resemblance to a fir tree more evident. In older cultures the surface expansion is thick and spreading and tends to cover the entire surface of the agar.

*Dextrose Agar Plates.*—Surface colonies thick and raised, 1 to 2 mm. in diameter in twenty-four hours. Under low power they show dense centers and irregular radiating peripheries made up of strands. Deep colonies punctiform, under low power dark, irregular, and spreading, made up of densely wound filaments. Bottom colonies spreading, up to 1 mm. in size. Under low power they show dark central masses with a spreading skein-like periphery looking like a tangled mass of hair. The agar is highly acid by the end of the first day. In forty-eight hours the surface colonies are thicker and heavier, up to ½ cm. in size, and by the end of four to five days they may be ¾ cm. in diameter. Later, after eight to ten days, the surface colonies are thick, dense, and opaque, with irregular branching margins, often quite large, 1 cm. in diameter. The original acid reaction gives way to alkali and eventually all the plates are alkaline and deep blue.

*Dextrose Agar Slant.*—Thick, dull white, abundant, raised growth in twenty-four hours with an acid reaction in the agar. In two to three days the growth is much thicker and heavier and the agar highly acid. The growth increases in breadth and thickness and eventually covers the medium. The acid reaction gives way to alkali and finally the agar becomes deep blue.

*Gelatin Plates.*—Colonies well-developed in forty-eight hours, round and regular, with branching edges, ½ mm. In thickly seeded plates the gelatin is liquefied in two days. Isolated colonies grow rapidly and by the end of three to four days are ¾ to 1 mm. in size, lying in cups of liquid gelatin about 2 mm. in diameter. Under low power the colonies show dense centers and distinctly feathery edges. Outside the colonies lies a rim of liquid gelatin. In five to six days the colonies are 3 to 4 mm. in size with the zone of liquid gelatin outside them about 1 cm. in diameter. Eventually the gelatin is entirely liquefied.

*Gelatin Stab.*—Growth appears in forty-eight hours along the line of inoculation with a distinct surface cupping, liquid gelatin lying at the bottom of the cup. The line growth shows feathery outgrowth like that seen in agar. In three to four days the liquefaction is transverse and gradually the gelatin is liquefied from above downward. After ten to twelve days the zone of liquid gelatin may be 1 to 2 cm. in depth. Eventually the entire mass of gelatin is liquefied.

*Blood-serum.*—Abundant, elevated, whitish growth in twenty-four hours. In two to three days this growth is much thicker and heavier and tends to spread over the surface. The serum is slowly softened and may show a partial liquefaction at the end of two to three weeks.

*Potato.*—Dry, elevated, abundant expansion in twenty-four hours. The growth rapidly becomes thicker and heavier and may be slightly pinkish or brownish in color. In old cultures it is thick, dry, and usually yellowish-white.

*Litmus Milk.*—Rapid growth, the milk being partially decolorized and acid in two to three days. The decolorization is complete in five to six days and the upper part of the medium begins to show considerable clearing. At the end of ten to twelve days the tubes show a clear fluid in the upper part and a white coagulum at the bottom. The litmus is completely reduced. Later the milk proteins are entirely digested.

*Broth.*—Turbidity in twenty-four hours with a skein growth in the upper part of the broth. Some sediment. In three to four days the skein growth is much more abundant and a considerable film forms on the surface. Later this film is precipitated and becomes part of the sediment.

*Peptone.*—Faint turbidity, well-marked skein growth and considerable sediment in two to three days. Later the skein growth is broken up and precipitated and a film forms on the surface. This is soon precipitated and forms part of the sediment.

**Fermentation Tubes.**—*Dextrose.*—Turbidity and skein growth in the bowl in two to three days. Reaction highly acid.

*Saccharose.*—Turbidity and skein growth in bowl. Reaction highly acid.

*Lactose.*—Skein growth and turbidity in bowl. Reaction neutral.

**Resistance.**—The vegetative rods are destroyed by heating to 55° C. for forty to sixty minutes. In dried blood destroyed by heating to 100° C. for two hours. Spore-free cultures may be destroyed by heating to 65° C. five and a half minutes, 75° C. three minutes, 80° C. one minute (Weil). Anthrax spores are highly resistant, but not so much so as the spores of some saprophytes. Dried on silk threads they survive for long periods, ten to twelve years (Aiello and Drago), seventeen years (Busson). In old gelatin cultures they have been found alive after eighteen and a half years (Székely). They are destroyed by hot air at 140° C. in three hours (Koch and Wolfhügel), by steam in three to ten minutes. They survive boiling water five minutes. They are killed by formalin steam in a partial vacuum at 60° C. in one-half hour. They also resist disinfectants, being destroyed by ½ per cent. sublimate solution in forty minutes (C. Fraenkel), by 10 per cent. solution in twenty minutes, by ½ per cent. hydrochloric acid sublimate in twenty minutes. Destroyed in three days by carbolic acid solution in 24 per cent. NaCl. Killed by lysol 5 per cent. in seven hours (Foth). Not killed by kreolin in thirty-five days (Hünermann). Destroyed in fifteen seconds by 0.2 per cent. chlorin water. Antiformin (5 to 10 per cent.) rapidly dissolves both spores and rods. Formalin 1 per cent. kills the spores in two hours, 2 to 5 per cent. in one hour, 10 to 20 per cent. in ten minutes (Hammer and Futler). Concentrated formalin (40 per cent. formaldehyd) destroys them in one to five minutes.

**Chemical Products.**—*Bacillus anthracis* produces substances which reduce litmus as shown in litmus agar cultures. It also reduces selenic acid to metallic selenium, red colonies appearing on agar and gelatin to which selenic acid salts are added (Scheurlein[12] Klett). In 10 per cent. peptone it produces $H_2S$. It produces acid in blood-serum (Behring) and acid in litmus milk whey (Petruschky[13]), the acidity of virulent cultures equalling 0.1 per cent. of decinormal acid. It is hemolytic for blood in both blood broth and blood agar plates, rabbit and sheep blood being attacked more than horse blood (v. Krogh[14]). By cultivating the organism in nutrose broth, Heyrowsky and Landsteiner[15] found a thermolabile hematoxin. In milk the fats and sugars are not attacked, but volatile acids, formic, acetic, and caproic, are produced from casein (Iwanow). Roger[16] states that *Bacillus anthracis* attacks glycogen both inside and outside the body, but sugars only in cultures. Mannius believes that the organism converts starches to sugar and gradually ferments the latter, lactic and acetic acid being formed (Napias). A diastatic ferment has been found by Fermi and a proteolytic ferment by Malfitano and Strada.[17]

**Pathogenic Properties.**—Anthrax or splenic fever occurs as a natural disease in cattle, horses, sheep, and a number of other species. Infection may be produced with pure cultures by subcuntaneous, intravenous, and intraperitoneal inoculation, by inhalation and by feeding. Mice, guinea-pigs, and rabbits are especially sensitive. In these animals subcutaneous inoculation produces fatal results in twenty-four to forty-eight hours, at autopsy the animals showing wide-spread jelly-like edematous infiltration, generalized enlargement of the glands, and enlargement of the spleen (acute splenic tumor). The disease is primarily a septicemia. The finest capillaries are filled with organisms, especially in the spleen and liver, the glomeruli of the kidneys, and the mucous membrane of the intestines. With organisms of low virulence the subcutaneous edema may be very marked and extensive. Infection can also be produced in both black and white rats, but not uniformly. Müller estimates that 79.4 per cent. black rats survive infection, 14 per cent. white rats, 23.4 per cent. black-white rats, 36.3 per cent. gray-white. When rats succumb they usually die on the third day.

Guinea-pigs, rabbits, and rats can be infected by feeding large quantities of spore material (C. Fraenkel), white mice also (Koch). The spores vegetate in the intestine, penetrate the mucous membrane, and cause a general infection. Mice, guinea-pigs, and rabbits may be

infected by inhalation (Buchner), the organisms penetrating the alveolar epithelium and producing a generalized septicemia.

Cattle are resistant to artificial infection despite their great sensitiveness to natural infection. Sheep are very susceptible, often dying in forty-eight hours. Swine are slightly sensitive to subcutaneous inoculation, not to feeding. Dogs may be killed by intravenous injection of large quantities. Birds are only slightly sensitive. Frogs and turtles resistant.

Infection in man by *Bacillus anthracis* is relatively common. Subcutaneous introduction of the organism produces lesions known as malignant pustule. These are found on the hands and forearms of people who handle animals suffering from anthrax, or especially the hides of such animals. Malignant pustule is very common on the face among the inhabitants of countries where anthrax is common in cattle. The course of the pustule varies greatly. It may be spread rapidly, giving rise to a fatal septicemia, or may be walled off by connective tissue, become chronic, and slowly heal. Infection of the eye is common in people who handle hides of anthrax animals, and pulmonary infection known as "wool-sorters' disease." In the latter, according to Eppinger, the spores are inhaled, develop into vegetative forms, travel along the lymphatics to the lungs and pleura, and produce an irregular pneumonia, with organisms in the sputum. This disease is practically always fatal. Subcutaneous infection may also occur through the use of shaving brushes made from the bristles of animals suffering from anthrax and not properly sterilized, or of bristles which have become accidentally contaminated.

The resistance of animals to the anthrax bacillus can be lessened by various methods. Thus hens and pigeons kept in cold water a long time develop infection more rapidly than controls (Pasteur and Joubert[18]), while frogs kept at high temperature lose their resistance (Gibier and Metchnikoff). Rats fatigued by running in a rotating drum are more sensitive than controls (Charrin and Roger). Rats fed on bread are more susceptible than rats fed on meat (Feser and K. Müller). The resistance of dogs, hens, and pigeons is lessened by long treatment with alcohol (Laitinen), while the resistance of pigeons is lessened by acute and chronic alcohol intoxication (Goldberg).

**Virulence.**—The virulence of cultures may be high, a few organisms or possibly a single bacillus producing fatal infection in small animals (Barber[19]). Usually the animals die from 1/20,000,000 of an oesa of a virulent culture just as regularly as from an entire oesa, the only difference being that the larger dose kills more rapidly, in twenty-four to thirty-six hours. The virulence may be lost on artificial cultivation. It may be brought back by animal passage, especially in pigeons (Metchnikoff), the virulence being increased for pigeons, guinea-pigs, and rabbits. Passage through rats and dogs also increases it.

**Antagonism.**—Several organisms destroy *Bacillus anthracis* in fluid and on solid cultures. The most important of these is *Bacillus pyocyaneus* which produces a soluble substance, pyoganase (Emmerich and Löw[20]), which dissolves the anthrax bacillus in the test-tube and has some curative action in infected animals, guinea-pigs, rabbits, and sheep (Fortineau). Pyocyanase is not destroyed by heating thirty minutes to 100° C. and is apparently not an enzyme. Streptococci are antagonistic to anthrax in mixed cultures on agar and gelatin (Emmerich) and act favorably in anthrax infections in rabbits. Staphylococci have slight antagonistic action "in vitro," but more marked action in the animal body, in rabbits, guinea-pigs, and mice infected with the organism. Certain animal sera are bactericidal to *Bacillus anthracis*, giving rise to a plasmolysis with contraction of the protoplasm, separation of the cell-wall, and a splitting up into spherical bodies. This action is most marked in rabbit serum (Pane), 1 c.c. killing 8000 organisms, and ceases after the serum has been heated to 57° C. twenty-four hours. Frog's lymph is highly bactericidal in the body, but not in the test-tube (Petruschky).

**Vaccination.**—Active immunity can be produced in animals only by the use of attenuated cultures as first shown by Pasteur, who cultivated the organism at 42° to 43° C. in broth, producing asporogenous strains of diminished virulence. The virulence is lost first for the least susceptible animals, being retained for the more sensitive. Vaccines of different powers of infecting animals can thus be obtained, this virulence being retained on successive cultivation. Pasteur's "premier vaccin" had no virulence for guinea-pigs and rabbits and was virulent for mice. His "deuxieme vaccin" was virulent for mice and guinea-pigs, but not for rabbits. The vaccines were actually forty-eight-hour broth cultures of these strains kept at 37° C. Sheep and cattle are vaccinated first with vaccin I and then with vaccin II. Similar vaccines have also been made by Chauveau[21] and by Koch, Gaffky, and Loeffler.[22] The results of treatment with vaccines are highly favorable. They produce almost complete protection of the animal and prevent great economic losses from the disease.

**Passive Immunity.**—Immune sera may be prepared in animals against *Bacillus anthracis* by active immunization and may be used for passive immunization. Favorable results have been reported from its use. Passive immunization has been employed especially by Sobernheim.[23] Sheep are first vaccinated by Pasteur's method, then given virulent cultures. The serum from such animals may be used for passive immunization or for passive in combination with active.

1. Pollender: Vierteljahresschr. f. gericht. u. öffentl. Med. v. Casper, 1855.
2. Brauell: Virchow's Arch., 1857, ii, 132. *Ibid.*, 1858, xiv, 432.
3. Koch: Beitr. z. Biol. d. Pflanz., 1876, ii, 303.
———: Mitth. a. d. K. Gsndhtsamte, 1881, i, 49.
4. Klett: Inaug. Dissert. Giessen, 1894. Deutsche tierärztl. Wchnschr., 1894, ii.
5. Schottelius: Centralbl. f. Bakteriol. [etc.], 1888, iv, 705.
6. Nakanishi: München. med. Wchnschr., 1900, xlvii, 187.
7. Prazmowski: Biol. Centralbl., 1888, viii, 301.
8. Lehmann: München. med. Wchnschr.,1887.
9. v. Behring: Ztschr. f. Hyg., 1889, vi, 117.
10. Roux: Ann. de l'Inst. Pasteur, 1887, i, 392. *Ibid.*, 1890, iv, 25.
11. Eisenberg: Centralbl. f. Bakteriol. [etc.], 1912, lxiii, Orig., 305.
12. Scheurlein: Arch. f. exper. Path. u. Pharmakol., 1896, xxxvii, 74.
13. Petruschky: Centralbl. f. Bakteriol. [etc.], 1890, vii, 609.
14. v. Krogh: Centralbl. f. Bakteriol. [etc.], 1910, liv, 188.
15. Heyrovsky and Landsteiner: Centralbl. f. Bakteriol. [etc.], 1907, xliv, Orig., 150.
16. Roger: Semaine med., 1893.
17. Malfitano and Strada: Compt. rend. Soc. de biol., 1905, lix, 2, 118; 120; 195; 197.
18. Pasteur and Joubert: Compt. rend. Acad. d. sc., 1877, lxxxiv, 900. *Ibid.*, 1877, lxxxv, 101.
19. Barber: J. Infect. Dis., 1909, vi, 634.
20. Emmerich and Löw: Ztschr. f. Hyg., 1899, xxxi, 1; 1901, xxxvi, 9.
21. Chauveau: Compt. rend. Acad. d. sc., 1884, xcviii, 73.
22. Koch, Gaffky, and Loeffler: Mitt. a. d. K. Gsndhtsamte, 1884, ii, 147.
23. Sobernheim: Ztschr. f. Hyg., 1897, xxv, 301. *Ibid.*, 1899, xxxi, 89.

### BACILLUS ANTHRACOIDES Hueppe and Wood

Originally obtained from the soil and described by Hueppe and Wood[1] as a motile non-pathogenic organism closely resembling *Bacillus anthracis*. Closely similar organisms and possibly the same have been found by Weinberg and Séguin[2] in gangrenous war wounds. The *Bacillus anthracoides* reported by Sheen and Klein[3] is probably the same species. We follow the description given by Weinberg and Séguin.

**Morphology.**—Bacilli resembling *Bacillus anthracis*, ends somewhat more rounded. The short forms resemble *Bacillus Welchii*, the long forms with spores *Bacillus œdematiens*.

**Motility.**—Undulatory movements in serous exudates and in cultures.

**Staining Reactions.**—Gram-positive in young cultures. In old cultures the degenerated forms are Gram-negative.

**Cultural Characters.**—Easily cultivated on ordinary media both in the presence and the absence of air.

*Martin's 2 Per Cent. Glucose Broth.*—Abundant turbidity with a gradual formation of a scum. This is precipitated and a new scum formed which is also precipitated, leaving a ring on the sides of the tube.

The medium has a sour odor.

*Martin's Broth without Sugar.*—The same type of growth but less abundant.

*Broth without Glucose Containing White of Egg.*—The same type of growth. The white of egg is not attacked.

*Agar Plate.*—Surface colonies look like colonies of *Bacillus anthracis*. Centers opaque, peripheries arborescent with interlacing filaments. Colonies sometimes whiter and more moist than with *Bacillus anthracis*.

*Gelatin Stab.*—Growth along line of inoculation with a halo of punctiform colonies. Progressive liquefaction of the gelatin with a surface scum.

*Litmus Milk.*—The milk turns alkaline and takes on a violet color. The casein is precipitated in fine particles and progressively digested. The serum is eventually yellow and amber-colored.

*Coagulated Serum.*—Grayish, humid, adherent colonies with progressive liquefaction of the medium.

*Potato.*—Abundant, moist growth turning yellow-brown in old cultures.

**Pathogenic Action.**—Weinberg and Séguin's isolations were non-pathogenic. The strains found by Sheen and Klein and also by Magrow were pathogenic for mice.

**Remarks.**—From the cultural reactions as given originally by Hueppe and Wood and later by Weinberg and Séguin *Bacillus anthracoides* is practically identical with *Bacillus cereus*. The strains labeled *Bacillus anthracoides* and sent to the writer's laboratory have always proved to be *Bacillus cereus*.

1. Hueppe and Wood: Berl. klin. Wchnschr., 1889, xxvi, 347.
2. Weinberg and Séguin: La gangrène gazeuse. p. 213.
3. Sheen and Klein: Lancet, 1915, i, 1292.

### Bacillus aërobius sepis Legros and Lecène

This organism was found by Legros and Lecène in a fatal case of gas gangrene in man. The same species was found by Manté in puerperal infections and in appendicitis.

**Morphology.**—Rod-shaped organism with rounded ends, straight or slightly curved, measuring 0.5 to 1 $\mu$ in width by 3 $\mu$ in length. It sometimes occurs as short chains.

**Motility.**—Active, undulatory.

**Spore-formation.**—Median spores appear in broth in two to three days.

**Staining Reactions.**—Stains with the basic anilin dyes. Gram-positive.

**Resistance.**—The spores resist boiling one-half hour.

**Cultural Reactions.**—This organism is a facultative anaërobe growing at temperatures between 18° to 43° C. Besson states that broth cultures give off an offensive butyric acid odor. Gelatin is rapidly liquefied. The organism grows well on agar and potato. It coagulates milk in fine granular flakes without changing the reaction. Coagulated serum is slowly liquefied. Odor of cultures fetid. No indol produced.

**Pathogenic Action.**—Subcutaneous inoculation in guinea-pigs produces a fatal gas gangrene in adult animals and a septicemia in young animals. Virulence easily lost. Increased by the simultaneous injection of lactic acid.

Legros and Lecène: Compt. rend. Soc. de biol., 1901, liii, 680.

### Bacillus piliformis Tyzzer

Isolated by Tyzzer[1] from a fatal disease of Japanese waltzing mice in which the liver shows small rounded nodules like miliary tubercles, varying in size from a small fraction of a millimeter to $2\frac{1}{2}$ mm. These nodules are opalescent and pale gray in color or opaque and yellow. Microscopically the nodules consist of necrotic tissue with an extensive infiltration of polymorphonuclear leukocytes. On the periphery of the nodules long slender bacilli occur within the living liver cells. The organisms lie parallel and are not present in the necrotic tissue, but may be in the phagocytic cells in the interior of the lesion. Spores are present as well as the slender bacilli. In some cases both bacilli and spores have been found in the bile and in the epithelium of the gall-bladder of the common and hepatic duct. The same bacillus has been found in the epithelium of the cecum and the first portion of the large intestine, in the glands of the mucosa, in endothelial phagocytes, in the interglandular connective tissue and in the walls of the blood-vessels in the mucosa. Similar organisms and spores are present in the fecal discharges mingled with the intestinal bacteria.

**Morphology.**—Slender, Gram-negative, spore-bearing, non-motile bacilli, varying greatly in length, thickness, and affinity for bacterial stains. The forms staining uniformly are about 0.5 $\mu$ thick and vary from 4 to 25 $\mu$ in length. The less intensely stained organisms appear slender and hair-like or thick and coarse. In such forms the deeply stained material is arranged in granules at regular intervals throughout the length of the organisms so that they have a beaded appearance. The deeply stained granules are small as compared with the faintly stained bands, except in overstained preparations in which they appear larger. With the Romanowsky dye these granules stain an intense red and the remainder of the organism bluish. The granules are frequently in pairs and this, together with the affinity for nuclear dyes, suggests that they are made up of chromatin material. Many of the organisms taper toward the ends, especially in the banded bacilli, the larger of which resemble slightly the fusiform bacilli of the alimentary tract. In the more deeply stained organisms the larger rods taper slightly, but the short rods have blunt and slightly rounded ends. The organisms are generally straight, but the larger forms may be bent, looped, or wavy. Long rods frequently show a centrally situated fusiform swelling which does not retain the stain after the manner of the spore. These may be involutions. Pairs of organisms attached end-to-end are present and occasionally a long unsegmented thread, but chains are not found. The

majority occur singly and when grouped have their long axes parallel. The spores are long, ellipsoidal in form, subterminal in their development. They vary from 3 to 4 $\mu$ in length, the average length being 3.3 $\mu$, and from 1.1 to 1.8 $\mu$ in thickness. The portion left unstained by ordinary dyes is cylindrical. With spore stains (carbolfuchsin followed by 2 per cent. sulphuric acid and counterstained with Loeffler's alkaline methylene-blue) there is a slender, cylindrical, centrally, situated stained portion surrounded by a clear zone and this in turn is enclosed by a bulging outer layer of varying thickness which takes the counterstain. The spores develop from the banded forms. The organisms become greatly thickened, divide into shorter segments, and the spores frequently occur in pairs. The portions of the organism forming the spore at first stain deeply but later, probably after the formation of a capsule, remain unstained. There is frequently a deeply stained granule near the end of the bacillus, opposite to that in which the spore is developing, the nature of which is not clear. The remains of the rod persist for a time but gradually disappear. The mature spores frequently show a slightly projecting knob at either end. The organism in general colors a purplish tint with Loeffler's blue, is not acid-fast, and is Gram-negative except that the granules in the banded form retain the violet stain. McJunkin's[2] modification of the Giemsa stain colors the granules deep red in marked color-contrast to the remainder of the bacillus, which is tinted bluish.

All attempts to cultivate the organism failed except for one possible growth in symbiosis with other bacteria. A slant of sugar-free agar showed in the water of condensation after three days under anaërobic conditions a great number of delicately curved bacilli with tapering ends and a few larger bacilli similar to those found in the liver lesions. Subcultures revealed this last organism with a streptococcus, but no successful cultivation without the streptococcus was accomplished.

**Pathogenic Action.**—The disease was produced in Japanese waltzing mice by feeding them diseased liver containing the organism but not in ordinary mice. Subcutaneous and intraperitoneal inoculation in ordinary mice, subcutaneous inoculation in guinea-pigs and rabbits, and intravenous inoculation in rabbits failed to produce the disease. Intravenous injection of salt solution suspensions of diseased liver into Japanese waltzing mice in small doses killed them in five days with lesions in the liver like those in the natural disease. Subcutaneous and intraperitoneal inoculation were usually without effect. The intravenous injection of small doses produced a pronounced immunity.

1. Tyzzer: J. Med. Research, 1917–18, xxxvii (n. s. xxxii), 307.
2. McJunkin: J. Am. M. Ass., 1915, lxv, 2164.

## GENUS 2. CLOSTRIDIUM Prazmowski

Anaërobes or micro-aërophiles. Often parasitic. Rods frequently enlarged at sporulation, producing clostridium or plectridium forms.

Type species *Clostridium butyricum* Prazmowski.

While the species of obligate anaërobes which have been accurately studied and described with sufficient clearness to be recognized by subsequent observers is small compared with the aërobes and facultative anaërobes, we have no reason to believe that they are actually less numerous. Such a large number of the common bacteria live well under anaërobic conditions that it is a difficult matter to obtain the obligate anaërobes by our available methods when they are mixed with the facultative anaërobes. Except in infectious processes in man and animals it is usually necessary to kill off the vegetative forms of the bacteria by heat, and, in consequence, we obtain only those anaërobes which have the capacity to form spores. Even with the best technic in the hands of skilled observers cultures seldom become pure because the spores of bacteria stick together so closely. The experience of the past few years has demonstrated that many cultures of anaërobes isolated by competent bacteriologists and studied with great care sometimes actually contain two or three different species. That the actual number of anaërobes is probably much larger than the forms described and named is indicated by the observations of Smith and Brown, who have found sixteen different varieties of obligate anaërobes in milk, only 2 per cent. of which represent well-recognized species.

Obligate anaërobes differ greatly in their sensitiveness to the presence

of atmospheric oxygen. Some develop readily when the amount of oxygen is slightly diminished, while others require almost its complete exclusion from the culture tubes. Even the strictest anaërobes grow when minimal traces of oxygen are present, as in the blood and tissues where it is always to be found. At the present time we usually speak of anaërobic conditions as partial tension conditions, referring to the diminished tension of the atmospheric oxygen as this is removed from our culture apparatus. Certain gases are indifferent to obligate anaërobes which will develop in atmospheres of hydrogen and carbon dioxid, or even in illuminating gas which contains large amounts of carbon monoxid in addition to other gaseous materials.

Other anaërobes are more sensitive and require an atmosphere of nitrogen, while still others grow best in a partial or complete vacuum.

The development of our knowledge of obligate anaërobes has been fraught with many difficulties, and up to the time of the last war only a few species were accurately defined. Probably the first pathogenic anaërobe to be described was Pasteur's *Vibrion septique* first isolated in 1861. Subsequently an organism was obtained by Koch, regarded by him as identical with Pasteur's species and named by Koch and Gaffky the *Bacillus of malignant edema*. A long controversy has existed as to the identification of the organisms described respectively by Pasteur and Koch and we may never know what organisms these men were actually working with. The experience of many observers during the past few years has cleared up this matter somewhat, and it is now generally accepted in France, England, and America that the true agent of malignant edema is represented by the *Vibrion septique* of Pasteur (*Clostridium septicum*), while Koch's *Bacillus of malignant edema* is thought to be the organism subsequently described by Metchnikoff as *Bacillus sporogenes*. This opinion is not shared by German bacteriologists, who still believe that malignant edema can be produced by the bacillus described by Koch. Several human cases of malignant edema have been reported in America and the organisms isolated are much more like Koch's organism than like Pasteur's. Other species, described with accuracy in the early days of modern bacteriology, are the *Bacillus of symptomatic anthrax* (*Bacillus chauvei* or *Rauschbrand*), the *tetanus bacillus*, *Bacillus aërogenes capsulatus* of Welch and Nuttall (the gas bacillus, *Bacterium Welchii*, etc.), and Novy's *Bacillus of Malignant edema* No. II. The great incidence of anaërobic infections during the war, especially in the condition known as gas gangrene, has led to a much more thorough study of obligate anaërobes than in previous years and authoritative papers on this subject have been published by von Hibler, Ghon and Sachs, Weinberg and Séguin, Muriel Robertson, Henry, Wolfe, and others in Europe, while a number of contributions have been made in America, notably those of Bull and Pritchet, Kahn, Hilda Hempel Heller, and Ivan Hall. At the present time we are therefore in the possession of a considerable body of knowledge about a limited number of obligate spore-forming anaërobes. Only a few non-sporulating anaërobes have been described, although we know that they are abundant in decomposing organic material and in infectious processes. Among these may be mentioned *Bacillus mortiferus* of Harris, *Bacillus fallax* and *Bacillus aërofœtidus* of Weinberg and Séguin, and various anaërobic streptococci and staphylococci.

**Classification.**—The classification of sporulating anaërobes for which the generic term *Clostridium* is now employed has been attempted along several lines. Thus v. Hibler has divided them into saccharolytic and proteolytic. The saccharolytic organisms are those which are especially active in the fermentation of the carbohydrates, while the proteolytic decompose proteins

energetically. Von Hibler has been followed by many of the English bacteriologists, notably by Henry. Among the saccharolytic anaërobes are Pasteur's *vibrion septique*, *Bacillus Welchii* (*Bacillus aërogenus capsulatus*), *Bacillus tertius* of Henry, and *Bacillus œdematiens* of Weinberg and Séguin. The proteolytic group includes *Bacillus tetani* of Nicolaier, *Bacillus putrificus* of Bienstock, *Bacillus sporogenes* of Metchnikoff, and *Bacillus histolyticus* of Weinberg and Séguin. This classification has been of considerable help in the study of war anaërobes. Proteolysis and saccharolysis are not opposing characters, however, and many anaërobes are proteolytic and saccharolytic. Adamson has divided the anaërobes by the type of sporulation, paying particular attention to the size and shape of the spore and its position in the vegetative rod. This scheme has been elaborated by Hall, who has given us the most complete and satisfactory classification of the anaërobes thus far devised. With certain minor changes Hall's classification may be adopted as follows:

*Anaërobes with central spores*

Type species *Clostridium butyricum* (Prazmowski).
" *Welchii* (Migula).
" *bifermentans* (Tissier and Martelly).
" L (Adamson) (Conradi and Bieling).
" *sarcemphysematodes hominis.*
" *egens* (Stoddard).
" *centrosporogenes* (Hall).

*Anaërobes with subterminal spores*

*Clostridium septicum* (Arloing and Macé).
" *œdematis maligni* (Zopf).
" *chauvei.*
" *of whale septicemia* (Nielson).
" *Novyi* (Migula).
" *of bradsot* (Nielson).
" *botulinum* (van Ermengem).
" *parabotulinum* (Seddon).
" *sporogenes* Metchnikoff.
" *parasporogenes* (McIntosh and Fildes).
" VI (von Hibler).
" VII (von Hibler).
" X (von Hibler).
" *fallax* (Weinberg and Séguin).
" *aërofœtidum* (Weinberg and Séguin).
" *histolyticum* (Weinberg and Séguin).
" *œdematiens* (Weinberg and Séguin).
" *multifermentans* (Stoddard).
Adamson's *central-spored anaërobe.*
Hempel's *proteolytic anaërobe.*

*Anaërobes with round terminal spores*

*Clostridium tetani* (Nicolaier).
" *pseudotetanicum* (Sanfelici).
" *putrificum* (Bienstock).
" *pseudotetanus* (Tavel).
" *tetanomorphum* (McIntosh and Fildes).

Type species *Clostridium tetanoides* A (Adamson).
" *tetanoides* B (Adamson).
" *tetanoides* E (Adamson).
" *spheroides* (Douglas, Fleming, and Colebrook).

*Anaërobes with distinctly oval spores*

*Clostridium tertium* (Henry).
" *cochlearium* (McIntosh and Fildes).
" S (Adamson).
" *spermoides* (Ninni).
" *tyrosinogenes* (Hall and Finnerud).

### CLOSTRIDIUM BUTYRICUM Prazmowski

A large number of non-pathogenic motile anaërobic butyric acid bacteria have been described and there are evidently many different strains of anaërobes marked by their capacity to induce butyric acid fermentation. The first member of this group was described by Pasteur[1] as *Vibrion butyric.* Subsequently butyric acid organisms were named *Clostridium pasturianum* by Winogradsky, *Bacillus amylobacter* by van Tieghem,[2] *Bacterium navicula* by Reinke and Berthold,[3] *Clostridium butyricum* by Prazmowski,[4] *Bacillus amylobacter* by Gruber,[5] *Bacillus butyricus* by Botkin,[6] *Bacillus saccharobutyricus* by v. Klecki,[7] *Granulobacter butyricus* by Beijerinck[8] and *Bacillus amylobacter* by Bredemann.[9] A similar organism was described by Grassberger and Schattenfroh[10] as a "motile butyric acid bacillus."

It is difficult to classify these butyric acid bacilli. They vary greatly in the reactions which have been attributed to them and in some instances the authors were probably not working with pure cultures. Henry[11] states that *Bacillus amylobacter* variety 1 of Gruber, *Bacillus amylobacter* of Bredemann, *Granulobacter butyricus* of Beijerinck, Grassberger and Schattenfroh's butyric acid bacillus, *Bacillus saccharobutyricus* of v. Klecki all induce stormy fermentation of milk and produce spores in it. According to Adamson[12] and the British Medical Research Committee.[13] *Clostridium butyricus* of Prazmowski, *Bacillus amylobacter* of Gruber, and Grassberger and Schottenfroh's motile butyric acid bacillus are alike and probably are identical with Pasteur's *Vibrion butyric.*

Adamson's description is as follows:

**Morphology.**—On agar Gram-positive rods in young cultures, Gram-negative after five to six days. Medium size or small, rounded ends, single, pairs, short chains. Filaments not common. Spores after five to seven days, oval, subterminal, or central. Spore no wider than bacillus producing it. Free spores elongated, oval, or almost rectangular with parallel sides. Vegetative rods motile.

**Cultural Characters.**—*Agar Colonies.*—Surface colonies small, circular, flat when young, thickened later. First transparent then creamy-white and opaque like a staphylococcus colony. Deep colonies small, irregular, and lenticular.

*Glucose Agar.*—Growth more rapid than on plain agar. Colonies larger, same type as on agar, circular, ultimately more opaque.

Deep stab shows much gas. Powerful odor of butyric acid.

*Dorset's Egg.*—No digestion or discoloration. Transparent moist surface film which later dries and fades. (Bacilli smaller than on agar. Spores abundant, produced slowly.)

*Egg Broth.*—Coagulation. No other change. Sour odor not offensive.

*White of Egg Broth.*—Much gas. Sour odor. No digestion, opacity, or precipitation.

*Broth.*—Scanty growth.

*Glucose Broth.*—Turbidity. Gas. Strong butyric acid smell.

*No indol.*

*Inspissated Serum.*—Little growth. No change in medium.

*Meat.*—Reddened. Gas, much turbidity also sour smell. Acid.

*Gelatin.*—No liquefaction.

*Milk.*—Acid. Clot. Some gas. No digestion.

*Sugars.*—Glucose, lactose, maltose, and saccharose fermented with acid and gas. No fermentation of mannite. More gas with glucose and maltose than with lactose or cane-sugar. Strong odor of butyric acid. Starch negative (positive M. R. C.).

Hall[14] gives the blood agar surface colonies as minute irregular moist drops with usually a mild hemolytic action while the deep colonies in 1 per cent. agar are globular, fluffy, and very diffuse. The organisms are less proteolytic than *Bacillus Welchii*. They produce no liquefaction or blackening of coagulated albumin. The addition of free iron does not result in blackening of brain media as with *Bacillus Welchii*. Gelatin not ordinarily liquefied; in some instances liquefied possibly from change in hydrogen-ion concentration. Hall's strain fermented glucose, lactose, saccharose, and salicin, but not glycrol or inulin.

**Non-pathogenic** to guinea-pigs.

Isolated from wounds only in one instance by Flemming. Obtained from putrefying meat by Adamson. Common in milk and soil.

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### Clostridium Welchii (Migula)

Discovered by Welch and Nuttall[1] in an autopsy on a case in which gas was present in the blood and tissues and named by them *Bacillus aërogenes capsulatus*. The same organism was obtained within a few months by Fraenkel[2] who called it *Bacillus phlegmones emphysematosæ*. It was described by Veillon and Zuber[3] as *Bacillus perfringens*. Named *Bacterium Welchii* by Migula. Owing to the fact that for many years this organism was believed to be almost the only species which could produce gas in the tissues it was usually called the "gas bacillus." It is frequently grouped with the butyric acid organisms because of its abundant production of butyric acid. Widely distributed in all parts of the world, both in the intestines of man and the animals, and in the environment, and a frequent cause of infections in man.

**Morphology.**—Large, encapsulated organisms with round or flat ends almost never square. They usually measure 0.7 to 0.8 $\mu$ in width by 4 to 6 $\mu$ in length. Uniformly enlarged on dextrose agar. They are arranged in single elements, pairs end-to-end and short chains. In older cultures the organisms show great variations in morphology. Chains of eight to ten elements are common, each element being a short fat bacillus. Whip forms about 0.5 $\mu$ in diameter and many micra long are also frequently observed. Peculiar swollen bizarre involution forms are also common in old cultures, especially in old fluid media or in coagulated serum. In the blood or tissues of infected animals or man the organisms are regular and uniform. On inspissated serum long filaments are common. Capsules are regularly present about the organism in the blood and tissues. They are thick and abundant and surround the organism with a uniform coating of mucinous substance. In cultures capsules are obtained most easily from milk and the water of condensation from agar slants, less regularly from broth, rarely from surface cultures on solid media.

*Spores* are formed regularly under certain conditions. They do not occur in the tissues and are seldom found in acid cultures. They occur but rarely in milk, their formation being inhibited apparently by the acid formed. They are present on blood-serum cultures, in alkaline sugar-free broth or in broth containing serum, egg-albumin, or meat. They do not often appear in sugar solutions. In cooked meat they may be present after five days

Fig. 155.—*Bacillus Welchii* from rabbit liver, stained by Huntoon.

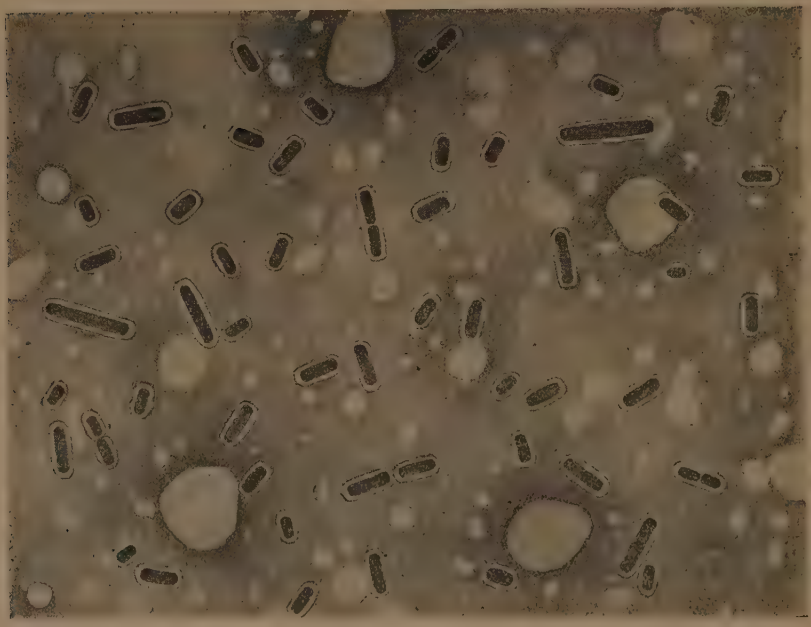


Fig. 156.—*Bacillus Welchii*, from heart blood of rabbit, stained by Hiss's method.

Formed rapidly in alkaline egg-albumin (Henry[4]). Spores oval with slightly flattened ends, central, a few subterminal, often large, taking up most of the bacillary body. They usually measure 0.5 by 1.5 $\mu$.

**Staining Reactions.**—The gas bacillus stains well with the ordinary anilin dyes, particularly with gentian-violet. In young cultures twenty-four hours old it is strongly Gram-

positive for the majority of the organisms, but there are always a few which decolorize. In older cultures, two to three days, the number of Gram-negative organisms increases and at the end of five to six days sometimes as many Gram-negative as Gram-positive organisms are present in films. It is usually believed that these Gram-negative forms represent old or dead organisms. They occur however in very young active cultures and may be old forms from the previous cultures, from which the growth was obtained. It is usually stated that *Bacillus Welchii* is partly Gram-positive and partly Gram-negative. It should be pointed out that this does not mean that the organism is on the borderline between retention and decolorization as with other species, but that many individual elements decolorize in a Gram-positive strain. In the tissues of infected animals or in human infections the organisms are strongly Gram-positive, and Gram-negative organisms, while occasionally present, are difficult to find.

Capsules are best stained by Welch's, Hiss's or Huntoon's method. In Welch's method the capsular substance may take a very delicate purple stain or a faint purple line may be stained about an unstained area of capsular substance in which the organisms lie. With Hiss's stain the capsular substance is often deeply stained. By Huntoon's method it is also deeply stained and is then seen to lie in a thick film or zone about the body of the bacillus.

**Cultivated** without difficulty under anaërobic conditions or at times under partial tension. Grows best at 37° C., slowly at 22° C., little or not at all below 12° to 15° C. Isolation possible at times directly from infected material. When associated with other organisms it may be difficult to obtain pure and is best gotten by the intravenous inoculation of rabbits. If the animals are killed in five to ten minutes and kept in a warm place a characteristic reaction develops in the blood and organs and the organisms may be cultivated from the heart's blood. Pure cultures may also be obtained on plates, preferably of blood agar. Sometimes pure cultures result from rapid transfer. In actively growing strains transfers may be made every ten to twelve hours and the "gas bacillus" will outgrow the organisms with which it is associated. The most satisfactory method is the use of deep, plain, or glucose agar shake cultures. The colonies are characteristic and may be transferred by small glass pipets or sterile platinum needles under sufficient magnification.

*Plain Agar Plates.*—Colonies develop slowly, appearing on the surface in twenty-four to forty-eight hours. They are usually circular, translucent, 1 to 2 mm. in size. Under low power they are circumscribed, with entire edges. Deep colonies small, barely visible in one to two days. Under low power lenticular. Older surface colonies are larger, opaque, and granular, with crenated edges. Other colonies may show very thick opaque centers and granular, slightly crenated margins, lobulated or typhiform. Some colonies are viscid and adherent to the medium. Deep colonies remain compact, opaque disks.

*Serum Agar Colonies.*—Like those on plain agar.

*Agar + 10 or + 12 to Phenolphthalein (Eyre's Scale) Smeared with Alkaline Egg-albumin.*—Young colonies semitransparent and opalescent, 2 mm. in diameter in twenty-four hours, 4 mm. in forty-eight hours. Under low power they show central raised granular areas surrounded by a narrow refractile rim (Henry). Older colonies show an opaque, central boss with a wider spreading slightly granular margin. After repeated subcultures colonies may become opaque and heaped-up like staphylococcus colonies. On a mixture of 10 per cent. gelatin and 2 per cent. agar plus alkaline egg fluid, colonies are speckled with dendritic processes which project down as roots into the medium (Henry). They appear white by reflected light, black by transmitted light.

*Plain Agar Slant.*—Moist, rather homogeneous layer in forty-eight to seventy-two hours, often with crenated or lobulated edges.

*Plain Agar Stab.*—Growth appears in two to three days as a distinct whitish line in the depths, often stopping abruptly just beneath the surface. Rarely a slight surface growth appears about the line of puncture, soft and homogeneous with lobulated or crenated margins.

Dark colonies in lead acetate peptone agar due to the production of $H_2S$ (Hall[5]).

*Dextrose agar colonies* appear on the surface in twenty-four to forty-eight hours, 1 to 2 mm. in size, translucent, and homogeneous. They are like those on plain agar, but usually somewhat larger, opaque, yellowish-brown, finely granular with frayed edges made up of individual bacilli. Deep colonies lenticular. The individual colonies are surrounded by gas bubbles and the agar turns acid. Sometimes gas collects between the agar and the bottom of the plate and all the agar may show an acid reaction. In old plates the colonies are larger, 4 to 5 mm. in size. The acidity may be permanent or give way to an alkaline reaction.

*Dextrose Agar Slant.*—Moist, homogeneous layer in twenty-four to forty-eight hours. Agar turns acid and bubbles of gas may appear in the water of condensation which becomes turbid. Acidity permanent or gives way to an alkaline reaction.

*Dextrose Agar Stab.*—Abundant growth in the depths along the line of inoculation. Agar turned acid and fragmented by bubbles of gas. Acidity usually permanent.

*Blood agar plates* containing 5 per cent. human, rabbit, or horse blood. Surface colonies appear in twenty-four to forty-eight hours, small, irregular, and transparent, 2 to 3 mm. in

size, surrounded by a narrow zone of hemolysis 6 to 7 mm. in diameter. The zones of hemolysis may increase in size, but do not coalesce. The colonies gradually become opaque. Deep colonies lenticular, also surrounded by a zone of hemolysis. Under low power the surface colonies are opaque, finely granular, and occasionally show distinctly fuzzy edges. The deep colonies may also appear as small fuzzy spheres (Herter[6]).

*Gelatin Plates.*—Colonies develop slowly, appearing in five to six days, 1 to 2 mm. in size. Under low power they are irregular and fuzzy. The gelatin may be slightly softened about the colonies.

*Gelatin Stab.*—Slow growth at 22° C. along the line of inoculation, appearing as a hazy line after five to six days, particularly in the lower part of the tube. Often no growth in the upper portion. Little or no surface growth. Gelatin slowly softened at this temperature. At 37° C. growth is very active and gelatin sometimes blackened and liquefied (Hall). Kahn[7] states that gelatin may also show gas production and complete liquefaction with a final pH of 6.4 after 8 months. The change in gelatin is best demonstrated by incubation at 37° C. for forty-eight hours and subsequent cooling in cold water. Kendall, Day, and Walker[8] believe that the softening of gelatin may be due to a change in the gelatin protein as a result of which solidification no longer takes place, and not to true proteolysis. There is no soluble gelatinase, and no change in titratable acidity.

*Loeffler's Blood-serum.*—Growth usually somewhat sparse, appearing in two to three days as a moist, homogeneous layer with lobulated or crenated margins. Serum not liquefied.

*Inspissated Serum Stab* (Kahn).—Large amount of gas, medium split into several fragments, pigment dark, more abundant than with any other of the non-proteolytic anaërobes. No digestion.

*Potato.*—Little or no growth on ordinary potato. On Wrzosek's potato vigorous growth may occur with abundant gas production (Simonds[9]).

*Robertson's Cooked Meat.*—Abundant gas production, liquid canary-yellow or orange-colored, beef itself brick-red. No blackening. Reaction acid, smell sour, no odor of putrefaction. Deposit of white, pearly, acicular crystals of tyrosin, soluble in ammonia. According to Kahn the fluid is turbid at first and clears later with a reduction of size in the particles of meat without loss in volume, pH 0.6 after eight months.

*Brain medium* slightly softened, not blackened or liquefied. No digestion. Blackened in the presence of an iron nail, due to the liberation of $H_2S$ (Hall).

*Solidified Egg Cube Broth.*—Much gas. Turbid fluid clearing later. No alteration in size of egg cube. pH after eight months 6.4 (Kahn).

*Modified Egg Water.*—Clotting, much gas. Turbid fluid clearing later. Clot becomes shrunken, hard, and caseous, with a copious, clear, watery fluid (Kahn).

*Alkaline Egg-albumin.*—Fine delicate network of clot in three to four days. Clot soft and friable. On the addition of a fermentable sugar a stormy fermentation occurs with a tough, spongy clot (Henry). According to Kahn the fibrinous network described by Henry does not occur.

*Dorset's Egg Medium.*—White, circular colonies sinking into the medium. No liquefaction or discoloration (Adamson[10]).

*Cooked Codfish Medium.*—Small amount of gas. No other reaction (Kahn).

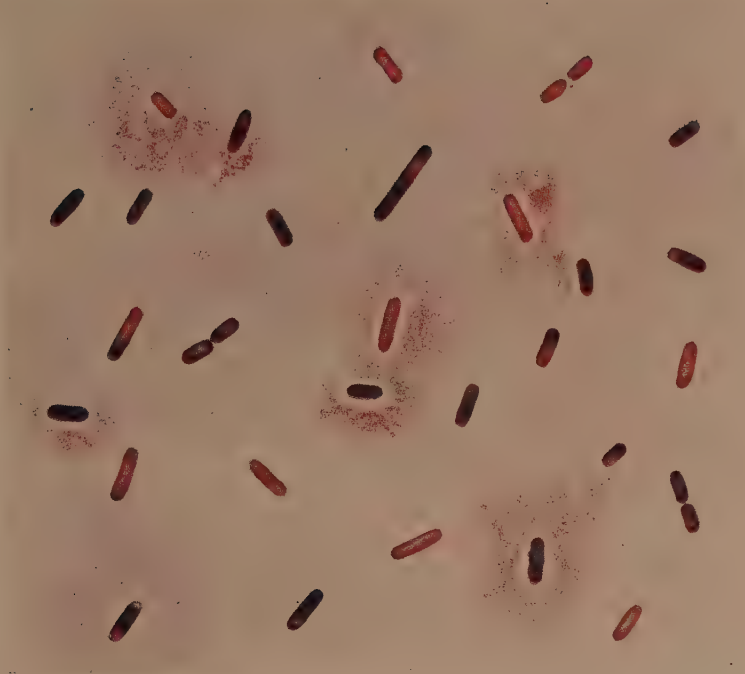
*Milk.*—Rapid acidification and coagulation with the production of large amount of gas, a characteristic stormy fermentation. The reaction may take place in twenty-four hours at 37° C. In a few days the clot shrinks somewhat and has a peculiar mouse-eaten appearance. Fluid at first turbid then clears. Coagulation of milk due to butyric acid. Wolfe and Telfer[11] have found that the acids in milk consist of 60 per cent. volatile acid (butyric acid, 60 per cent., acetic acid, 40 per cent., with traces of formic acid) and 40 per cent. fixed acid, chiefly lactic acid. Gas largely hydrogen, 90 per cent. at first, later 62 per cent., and finally 25 per cent. (Wolfe and Harris[12]). Balance carbon dioxid. Gas derived from splitting of lactose. Little or no proteolysis. According to Wolfe considerable quantities of amino-acid and small quantities of ammonia are produced in milk.

*Bromcresol-purple Milk.*—Stormy fermentation, pH 5.2 after eight months.

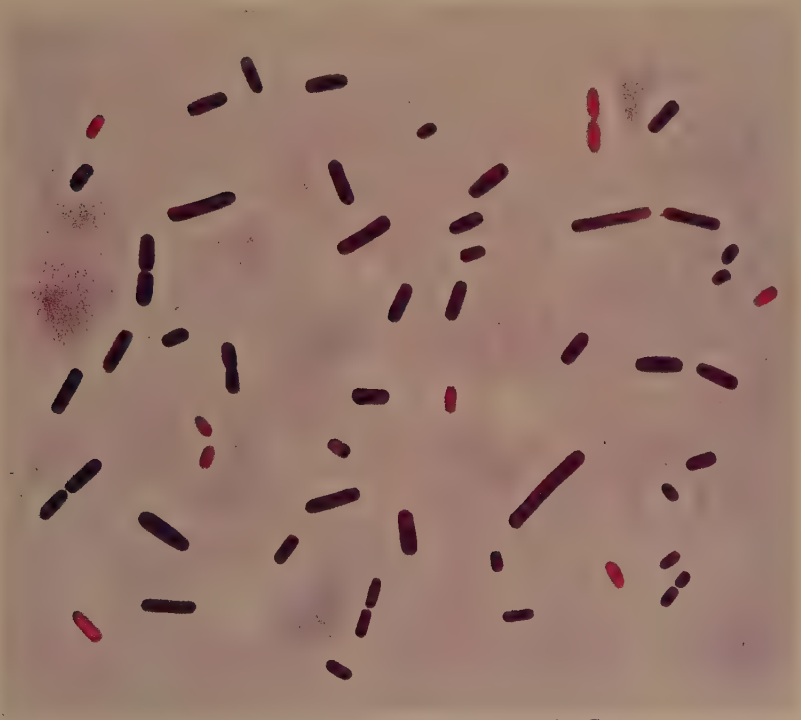
*Broth.*—Diffuse turbidity in two to three days with a whitish sediment. No scum. Growth may be sticky.

*Peptone.*—Growth much the same as in broth but somewhat more scanty. Growth rarely sticky.

*Dextrose Fermentation Tube.*—Growth abundant in the bulb as a diffuse turbidity and sediment. It extends into the closed arm and considerable gas collects in two to three days. Sometimes the gas may occupy the entire length of the closed arm. It consists of hydrogen and carbon dioxid usually having the composition $\frac{H}{CO_2} = \frac{2-3}{1}$. The broth has an acid reaction and may be mucoid. The acids consist chiefly of butyric and acetic acid with trace of formic. The addition of a piece of sterile rabbit kidney considerably enhances the growth of the gas bacillus in Smith fermentation tubes. In 20 per cent. dextrose peptone solution Wolfe and Telfer have shown that 49 per cent. of the acid is acetic acid and 41 per cent. butyric.



Bacillus Welchii, from agar culture from rabbit liver, stained by Gram.



Bacillus Welchii, from rabbit liver, stained by Gram.

*Other Sugars.*—The first recognition that strains of *Bacillus Welchii* differ in regard to their action on carbohydrates is due to Simonds,[13] who divided the organism into four groups:

Group I ferments inulin and glycerin.
Group II ferments glycerin, not inulin.
Group III ferments inulin, not glycerin.
Group IV does not ferment glycerin or inulin.

The strains of gas bacillus were subsequently classified by Henry who recognized 4 types. Type I ferments glucose, levulose, galactose, saccharose, maltose, lactose, raffinose, mannose, starch, dextrin, and glycogen. This type corresponds to Simonds' Group IV.

Type II ferments these carbohydrates and, in addition, glycerin. This type corresponds to Simonds' Group II.

Type III ferments these carbohydrates and, in addition, inulin. This type corresponds to Simonds' Group III.

Type IV ferments these carbohydrates and, in addition, inulin and glycerin. This type corresponds to Simonds' Group I.

Mannite, dulcite, and salicin are not ordinarily fermented, but according to Hall some strains may ferment salicin. Kahn has described strains fermenting also xylose, melezitose, and inosite. In the opinion of Kendall, Day, and Walker, *Bacillus Welchii* attacks the hexose sugars, glucose, fructose, mannose, and galactose energetically, but not the hexose alcohols, sorbitol, mannitol, and dulcitol. The aldose configuration CHO affords a point of attachment, not the alcohol configuration $CH_2OH$. With sucrose there is possibly a preliminary hydrolysis. Humphreys has pointed out that *Bacillus Welchii* produces the aldehyd acrolein in glycerol broth. After eighteen hours this is sufficient to inhibit growth and kill the organisms.

**Proteolysis.**—*Bacillus Welchii* is essentially a saccharolytic organism, its proteolytic powers being limited. It induces a softening or liquefaction of gelatin, especially when grown at high temperatures, 37° C. It does not cause a true proteolysis of gelatin with degradation products. It does not produce indol. Gas appears in cooked meat, but this may be due to the sugars or amino-acids. Wolfe and Harris state that a smaller amount of gas is found in sugar-free peptone water and an appreciable amount of amino-acid and ammonia are produced. A slight proteolysis may also occur in sugar-free alkaline casein with some amino-acid and ammonia. In erepton (meat digested by trypsin and erepsin) consisting chiefly of amino-acid, there is an increase of ammonia and a destruction of the amino-acid. The butyric acid found in protein solutions may not come primarily from the proteins but from amino-acids.

**Thermal Death-point.**—Vegetative rods killed at 57° to 60° C. in twenty to thirty minutes. Spores resist temperatures of 80° to 90° C. for some time and even boiling for a few minutes. Practically always killed by boiling ten to fifteen minutes.

**Pathogenic Action.**—Freshly isolated cultures are virulent to small animals, guinea-pigs, pigeons, and sparrows (Welch[14]). Following subcutaneous inoculation a local necrosis develops with a good deal of gas in the adjacent tissues. Frequently a slough forms, the animals dying in four to five days. At autopsy there is an abundant bloody edema which microscopically shows the organisms and a few leukocytes. Gas bacilli are rarely present in the blood-stream and there is no general invasion of the body before death. Rabbits and mice are less susceptible than guinea-pigs or pigeons. Old cultures are apt to have little or no virulence. The most marked property of the gas bacillus is its ability to produce gas in the tissues after death. This is best demonstrated by the intravenous inoculation of rabbits which are killed after five to ten minutes and then kept a few hours in a warm place. At autopsy the changes are characteristic. The blood is filled with gas bubbles; gas blebs are found everywhere in the organs and tissues, especially in the fascias between the layers of muscle and sometimes in the serous cavities. The liver shows a characteristic reaction and is literally shot to pieces with gas blebs. The organism can usually be found in the heart's blood in pure culture. In man *Bacillus Welchii* is the principal if not the sole cause of the pathological condition in which gas is present in the blood and organs. The amount of gas may vary from a few bubbles to extensive emphysema of the organs and tissues (so-called foamy organs). The gas may appear within an hour after death (Welch), but it is not entirely clear whether gas actually accumulates during life. The organism grows so rapidly in injured tissues that the presence of gas during life must be regarded as a possibility, even though the rapid development after death explains the large amount of gas so frequently found. Various areas may serve as a portal of entry such as the infected uterus (Dobbin[15]), where the fetus itself may be emphysematous and the uterus distended with gas, or the urinary passages like the urethra and bladder. The invasion may be from the intestine (Howard[16]) with or without definite intestinal lesions. The gas bacillus may cause pneumoperitonitis with or without perforation. It is a frequent cause of infections about the bile-ducts and in the liver, especially the so-called foamy liver. Interstitial emphysema of the gastro-intestinal, genito-urinary, and biliary tracts is practically always due to the action of this organism. Emphysema of the vagina has been traced by Lindenthal[17] to it. It is also found in lung infections (Welch and Flexner[18]), in pulmonary gangrene (Rist[19] and Guillemot[20]), in pneumo-

thorax without perforation (Levy[21]). During life the gas bacillus has been obtained from the blood by Gwyn.[22] It has also been obtained from meningitis by Howard[23] and by Hitschmann and Lindenthal[24] and from cavities in the brain by Reuling and Herring[25] and by Howard. Possibly the most frequent type of infection by *Bacillus Welchii* is emphysematous gangrene following trauma or following surgical operation. The emphysema may be limited to the neighborhood of the wound or may spread and involve large areas of the adjacent subcutaneous tissues. Emphysematous gangrene may occur in almost any part of the body, but is more frequent in the extremities. It is seen most often after gunshot wounds and compound fractures. The infection may spread quickly from the portal of entry and give rise to a generalized emphysematous gangrene which is rapidly fatal. During the late war *Bacillus Welchii* was the most frequent cause of wound infections (Henry). In many instances it was associated with other pathogenic anaërobes like *Bacillus tetani*, with non-pathogenes like *Bacillus sporogenes*, or with aërobic organisms. In other cases it was the only species isolated.

**Toxins.**—True toxins were first demonstrated in cultures of the gas bacillus by Bull and Pritchett[26] who found that filtered cultures were virulent to animals, especially to guinea-pigs, and that the serum of animals appropriately treated contains an antitoxin. The toxin is produced early in broth cultures, best in broth containing pieces of sterile tissue like the rabbit's kidney. It has a strength such that 0.3 to 5 c.c. kills a guinea-pig of 250 to 300 gram weight in four to five days. Rarely more virulent strains are found, in which 0.1 c.c. of a broth culture is a fatal dose for a guinea-pig. By the immunization of rabbits an antitoxic serum may be prepared of such a strength that 0.5 to 1 c.c. neutralizes 1 to 4 c.c. of toxic culture. An antitoxic serum can also be obtained by the immunization of horses, and has been used with considerable success in the treatment of wounds infected with *Bacillus Welchii*.

**Hemotoxin.**—*Bacillus Welchii* produces a true hemotoxin, as first shown by Ford and Lawrence.[27] This dissolves the red blood-corpuscles of various species of animals like rabbits and guinea-pigs and human corpuscles. It has a strength such that .025 c.c. will dissolve 1 c.c. of a 5 per cent. rabbit's blood suspension. This hemotoxin is independent of the acids produced by the organism, is destroyed at temperatures from 55° to 60° C. in thirty minutes, digested by pepsin and pancreatin and may be precipitated by ethyl alcohol. It has also been shown by Ford and Williams[28] that an antihemotoxin can be obtained by the immunization of rabbits, 0.001 c.c. of serum neutralizing a single unit of hemotoxin. The results obtained by these authors have been recently confirmed by Henry.[29]

**Distribution.**—*Bacillus Welchii* is one of the most widely distributed organisms known. It occurs in the intestinal tract of man, both adults and children, and in practically all the domestic animals, horses, cattle, swine, goats, dogs, cats, rabbits, guinea-pigs, and rats. It also occurs in the feces of wild animals, lions, tigers, and wolves. It occurs occasionally in shell-fish. It has been isolated frequently from soil, especially from garden earth, from dust, from water and sewage. It is practically always present in milk, frequently in association with other anaërobes.

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### CLOSTRIDIUM BIFERMENTANS (Tissier and Martelly)

Described originally by Tissier and Martelly[1] as *Bacillus bifermentans sporogenes* and obtained by them from putrefying meat. Subsequently found in gas gangrene by Weinberg and Séguin,[2] who gave it the binomial name *Bacillus bifermentans*, by Hilda Hempel Heller,[3] by Tissier,[4] and by the British Medical Research Committee.[5] Found in both acute and late cases. The present description follows Tissier and Martelly's original paper, with some details from subsequent workers.

**Morphology.**—A fairly large organism measuring 0.8 to 1 $\mu$ in breadth by 5 to 6 $\mu$ in length. Chains and filaments of five to six elements are formed in liquid media. Ends square or very slightly rounded. Rarely curved forms. Non-motile.

Spores formed early, in twenty-four hours in sugar-free media, later in sugar media. They form in the middle of the organism which does not swell or enlarge at sporulation.

Spores oval or oblong and measure 0.5 by 1.5 $\mu$. May also be subterminal (M. R. C.). Free spores slightly barrel-shaped (Hall[6]). Spores resist boiling one and a half minutes.

Stains well with the usual anilin dyes. Gram-positive.

**Cultural Characters.**—Cultivated only under strict anaërobic conditions at temperatures from 22° to 37° C., best in sugar media. Surface colonies round or crenated. Deep colonies lenticular or irregular without filamentous outgrowths.

*Deep Glucose Agar.*—Colonies begin to appear in twenty-four hours, regular, grayish-white. Well-developed in forty-eight hours. In about three days gas of a disagreeable odor is produced. Colonies are originally lenticular. After one to two weeks they show embossments, which appear as offshoots from the early colonies and often form a kind of crown about a central nucleus. They never become filamentous or hairy. Deep 1 per cent. infusion-agar colonies granular, diffusely opaque globules tending to break up as they become older (Hall).

*Deep Glucose Gelatin.*—Colonies like those in deep glucose agar. The gas appears slowly and after a month the gelatin is softened and liquefied.

*Gelatin* completely liquefied in forty-eight hours (Kahn[7]). Small amount of gas. Clearing after thirty-five days. Dirty-gray residue. pH 7.0 after eight months. Kendall, Day, and Walker[8] report that this species produces a soluble proteolytic enzyme which induces liquefaction in a solution of gelatin in 0.5 per cent. phenol. No increase of ammonia. Some increase of amino-nitrogen content indicating gradual breaking of protein-tie in gelatin molecules, with a coincident liberation of free $NH_2$ groups as mono-amino acids and polypeptids.

*Plain Broth.*—Moderate development. Turbidity and viscid sediment in forty-eight hours.

*Glucose Broth.*—Typical development of a mucoid mass adhering to the bottom of the tube. On violent agitation this mass floats to the surface like a colony of molds.

*Milk.*—Rapid development. In five hours the casein is precipitated with the formation of small fine clots, the fat lying on top. Serum yellow-white, casein rapidly and completely digested. Slight gas production (Hall).

*Bromcresol-purple Milk.*—Soft granular clot, no gas (Kahn). Digestion of three-fourths of clot. Fluid clears. pH after 8 months = 6.5.

*Blood Agar.*—Surface colonies irregular, ameboid, or lace-like, spreading or round like dew-drops (Hall). Blood hemolyzed.

*Cooked Meat.*—Blackened with gas production. Gas putrid. Broth turbid, then clears. Feeble proteolysis, about one-quarter the protein being dissolved after 8 mo. (Kahn). Faint putrefactive odor. No tyrosin or other crystals. pH after 8 months = 6.8.

*Solidified Egg Cube Broth.*—Reaction characteristic. Fluid cloudy with gas bubbles. Edges of cube slightly attacked. Stringy mucoid type of digestion with slimy elastic end-product (Kahn). No reduction of total volume of albumin. Broth clears after thirty days. pH after 8 months 6.8 (Kahn).

*Robertson Alkaline Egg Fluid.*—Gas production with a thickening of the medium which is later clotted and digested to a clear watery fluid. About one-quarter the egg digested. pH 6.9 after 8 months (Kahn).

*Modified Egg Water* of Kahn.—Soft clot, thick. turbid fluid with gas production. Clot flaky and loosely organized. Digestion of about one-quarter the egg (Kahn).

*Inspissated Serum.*—Gas production and blackening of medium with digestion of one-half its bulk. Clear watery fluid after ten days. Fluid acid after eight months pH 6.8 (Kahn).

*Cooked Codfish Medium.*—Gas production and turbidity. Medium turned dark slate-gray color. Feeble proteolysis, one-quarter the protein's digested, pH 7.1 after eight months (Kahn).

*Protein* substances, albumins, etc., digested by means of a diastase of the type of trypsin.

*Fibrin* decomposed with the production of indol, hydrogen sulphid, proteoses, amines, leucin, tyrosin, fatty and aromatic acids, and ammonia.

*Proteoses* decomposed with the formation of indol, hydrogen sulphid, amino-acids and ammonia.

*Fats* emulsified and saponified in cultures by the organism which apparently secretes a lipase.

*Bacillus bifermentans* attacks glucose actively, producing acid and gas. In a nitrogenous solution containing 1.5 sugar at the end of ten days the sugar amounts to but 0.8 per cent. No alcohol is found. Acetic and butyric acid are both produced, but no lactic acid. It produces acid and gas also from glucose, levulose, and maltose, also glycerol (Hall). Produces acid alone from galactose, saccharose, lactose, raffinose, xylose, salicin, and inulin. Sugar-free broth not fermented (Hall).

**Non-pathogenic** for laboratory animals. Non-toxic. Agglutinins against it may be produced in rabbits (M. R. C.).

Weinberg and Séguin regard *Bacillus bifermentans* as saccharolytic in that it ferments glucose, levulose, and maltose, and proteolytic in that it attacks gelatin, casein, egg-albumin, and coagulated serum like *Bacillus sporogenes*, with the production of a putrid odor. These authors consider that it is closely related to *Bacillus Welchii*, but with proteolytic properties like *Bacillus sporogenes*. Tissier and Martelly conclude that it is closely related to *Bacillus sporogenes* and one of the chief agents of putrefaction.

1. Tissier and Martelly: Ann. de l'Inst. Pasteur, 1902, xvi, 865.
2. Weinberg and Séguin: La gangrène gazeuse. 1916, p. 128.
3. Heller, Hilda Hempel: J. Infect. Dis., 1920, xxvii, 385.
———: J. Bact., 1922, viii, 1.
4. Tissier: Ann. de l'Inst. Pasteur, 1916, xxx, 681. *Ibid.*, 1917, xxxi, 161.
5. Rep. Brit. Med. Research Comm., No. 39.
6. Hall: J. Infect. Dis., 1922, xxx, 445.
7. Kahn: J. Med. Research, 1922, xliii, 155.
8. Kendall, Day, and Walker: J. Infect. Dis., 1922, xxx, 40.

### CLOSTRIDIUM L (Adamson)

Isolated as Bacillus L from a war wound by Adamson. Resembles *Bacillus Welchii* in morphology, but differs in cultural reactions.

**Morphology.**—Large, non-motile, Gram-positive bacillus with square cut ends. Larger or as large as *Bacillus Welchii*. Straight or curved. Isolated or chains and filaments. Spores scanty, oval, subterminal, and central, after five to six days on agar.

**Cultural Reactions.**—*Agar* growth slow. Colonies flat, moist, semitransparent, opaque in the center. Margins irregular, indented with fine prolongations. Slant agar colonies coalesce to form moist semitransparent growth.

*Alkaline Agar.*—Colonies smaller. Growth slower (bacilli more slender with few spores).

*Glucose Agar.*—Colonies more regular than on plain agar. Central zone less opaque than margins. (Bacilli large, in chains, beaded. No spores).

*Glucose Agar Stab.*—Line growth below surface, threads penetrating medium. No gas.

*Neutral Red Glucose Agar.*—No change in color or fluorescence.

*Dorset's Egg Medium.*—Small surface colonies, not sinking; no softening or discoloration.

*Egg Broth.*—Slow clot.

*Brain Medium.*—No gas production. No blackening. Pinkish color. No digestion. (Old organisms beaded.)

*Inspissated Serum.*—Scanty growth. Colonies transparent and gray-white. No erosion of surface. No liquefaction.

*Broth.*—Scanty growth with precipitate.

*No indol.*

*Gelatin.*—Liquefaction in three to four days. Deposit, five to six days. Upper layer clear.

*Milk.*—No acid or gas. Slightly alkaline white flocculent precipitate of casein, three to four days. No digestion. Acid in glucose and maltose. No gas. Lactose at first gave acid not later. No fermentation of saccharose or mannite.

*Meat not digested.*

Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.

### CLOSTRIDIUM SARCOEMPHYSEMATODES HOMINIS (Conradi and Bieling)

Found by Conradi and Bieling in many cases of gas gangrene. Possibly a mixture of *Bacillus Welchii* and *Bacillus sporogenes*.

Conradi and Bieling: München. med. Wchnschr., 1916, lxiii, 178.

### CLOSTRIDIUM EGENS (Stoddard)

Reported by Stoddard as *Bacillus egens* from a fatal case of gas gangrene at a Casualty Clearing Station in France. Closely resembles *Bacillus Welchii*, from which it differs by its action on milk and by its pathogenic properties.

**Morphology.**—About the same size and shape in young cultures as *Bacillus Welchii*, showing a well-defined capsule in the animal tissues and in broth, easily demonstrated by Welch's capsule stain. In older cultures this organism produces long thread-like forms which are especially marked in the condensation water of egg slants and on glucose agar. They are long, tortuous, five to ten times the length of *Bacillus Welchii* and may grow to a length one to three times that of an oil immersion field.

**Staining Reactions.**—Strongly Gram-positive in young cultures (twenty-four hours), but in forty-eight-hour cultures half the organisms are Gram-negative. The long threads are Gram-positive. In cultures four to five days old nearly all the bacilli except some of the long threads are Gram-negative and many small, non-refractive, swollen, nearly spherical organisms are present.

**Motility.**—Negative.

**Spores** not found.

**Cultural Reactions.**—*Bacillus egens* is a fairly strict anaërobe, no aërobic growths occurring on plates or slants.

*Glucose Agar Plates.*—Colonies on the surface in twenty-four hours are 0.5 to 2 mm., flat, round, with sharply defined edges, bluish-gray by transmitted light. Under low magnification by hand lens the colonies show fine grayish grating (striations) and reddish-purple, green, iridescent tints are visible. Older colonies are somewhat larger, but maintain the same morphological characters. Deep colonies are small, $\frac{1}{3}$ mm., opaque, white, lenticular, without processes. Both superficial and deep colonies are quite different from *Bacillus Welchii* colonies.

*Egg Slants.*—Colonies circular with sharp, regular outlines, pale gray in twenty-four hours, but later light yellow in color. Surface smooth, even, and shiny. Young colonies are 1 to 2 mm. in size, older (two to three days) 4 to 5 mm. The egg medium is not blackened or softened.

*Blood Agar.*—Deep colonies become brown in two to three days with small central dark brown nuclei. Surface and deep colonies show definite hemolytic zones in twenty-four hours.

*Milk.*—No gross changes in less than twelve days and usually not for fifteen to twenty days when an acid clot is slowly formed with little or no gas. The whey is gray and cloudy; no digestion of the casein. No stormy fermentation characteristic of *Bacillus Welchii*.

*Chopped Meat.*—A few gas bubbles but no digestion, blackening or other color production.

*Glucose Bouillon.*—Diffuse cloudiness which persists and a fine sediment.

*Plain Bouillon.*—Faint diffuse cloud.

*Fermentations.*—Glucose in serum water is fermented actively and vigorously, marked acid and gas formation occurring in twenty-four hours. Maltose and saccharose show slight acidity and gas in twenty-four to forty-eight hours. Lactose shows only a trace of acidity. Galactose and raffinose are fermented like saccharose. No change in mannite, dulcite, inulin, glycerol, or salicin.

**Proteolytic Action.**—*Bacillus egens* softens living muscle in infected animals, but in cultures has no proteolytic effect on casein, coagulated egg-albumin, cooked muscle, or coagulated horse serum. No indol production and no noticeable odors.

**Pathogenic Action.**—*Bacillus egens* is highly pathogenic for guinea-pigs and somewhat less so for rabbits. In twenty-four-hour broth cultures $\frac{1}{10}$ c.c. is fatal to guinea-pigs, but after passage through these animals the virulence was markedly increased, 0.02 c.c. of a twenty-four-hour glucose broth culture being fatal in thirty hours. For rabbits the toxicity is less, 2 c.c. of a twenty-four-hour glucose bouillon culture being fatal in two days. At autopsy the animals show extensive and complete muscle destruction at the site of inoculation in the thigh, muscle destruction at distant points in the abdominal wall or chest, or in the opposite leg. In addition there is a spreading gelatinous edema saturated with thin fluid and gas bubbles in the subcutaneous tissues. The infection spreads to the peritoneal cavity and to the blood-stream.

Stoddard: J. Exper. M., 1919, xxix, 187.

### CLOSTRIDIUM CENTROSPOROGENES (Hall)

Isolated by Hall[1] as *Bacillus centrosporogenes.*

**Morphology.**—Large Gram-positive rods occurring singly, in twos, in short chains and even in long filaments. Spores formed early in all media, especially in those devoid of sugar. Central in position and do not swell the rod. Oval, free spores abundant in old cultures. Vegetative rods highly motile in young cultures, non-motile in old cultures.

**Colonies.**—Surface colonies on blood agar minute hemolytic dew-drops that rapidly become more opaque and yellowish in color, spreading where well separated to a diameter of 1 to 2 mm., then appearing almost coliform. Deep agar colonies vary with water content and consistency of the medium. In 1 per cent. agar they are large globular fluffs with a central nucleus when young and several when older. They resemble colonies of *Bacillus sporogenes.*

**Actively Proteolytic.**—Coagulated egg-albumin, brain, and meat media with less than an excess of fermentable sugar are digested and blackened. Gelatin is liquefied and blackened. Milk slowly coagulated and liquefied with slight gas production.

Glucose broth cultures sometimes mucoid, sometimes not. Old deep brain cultures show round balls of white crystals that are thought to be leucin or tyrosin or possibly a mixture of both. (Like that described by Bertholet,[2] for *Bacillus sporogenes* of Metchnikoff).

Glucose fermented. No action on lactose, saccharose, salicin, glycerol, inulin, and sugar-free media.

**Non-pathogenic** to rabbits and guinea-pigs.

1. Hall: J. Infect. Dis,. 1922, xxx, 445.
2. Bertholet: Ann. de l'Inst. Pasteur, 1909, xxiii, 85.

### CLOSTRIDIUM SEPTICUM (Arloing and Macé)

First obtained by Pasteur from the blood of animals dead of anthrax and called *Vibrion septique.* Named *Bacillus septicus* by Arloing and Macé. About the same time an anaërobic species was obtained by Koch and regarded by him as identical with Pasteur's organism. The usual opinion among French, English, and American bacteriologists is that Koch had in his hands the organism now called *Bacillus sporogenes* of Metchnikoff. This opinion is not shared by German bacteriologists who regard Koch's organism as the true bacillus of malignant edema.

Organisms corresponding to the type of *Vibrion septique* of Pasteur and the malignant edema bacillus of Koch have been isolated repeatedly by observers in many countries, among whom may be mentioned Chauveau and Arloing, Braatz, Bremer, Hoegh, Nekam, Campenon, G. Marchand, Ménereul, Monad, Hove, Pagmotti, Tubby and Wright, Mason, Eisenberg, v. Hibler, Haemig and Silberschmidt, Brabek, Silberschmidt, Gould, Ghon and Sachs, and de Besche.

The matter of the identification of the two organisms was first cleared up somewhat by Ghon and Sachs in 1903–4, who isolated an anaërobic bacillus from the gaseous gangrene of the abdominal wall in a case of ruptured appendicitis. Their organism was identified by comparing the original strain isolated by Pasteur and sent by Metchnikoff to Grassberger and Schattenfroh with which it was identical. Ghon and Sachs description would fit also the original description of Koch's bacillus which is now lost. Ghon and Sachs bacillus was regarded by them as the same as Koch's organism, and they therefore selected the name *Bacillus œdematis maligni* as the correct designation. This organism was non-proteolytic. Von Hibler, however, in 1908, did not accept Ghon and Sachs contention, but considered a proteolytic strain isolated by himself as the true malignant edema bacillus. During the war several cases of fatal gas gangrene were observed by Weinberg, who obtained organisms corresponding definitely to Pasteur's *Vibrion septique* as preserved in the Pasteur Institute, and two similar strains were found by Hilda Hempel Heller in Muriel Robertson's laboratory. According

to Weinberg and Séguin it was definitely isolated thirteen times during the war, and in addition a number of times in animals as in pigs, by Karl Meyer.

The following description is taken chiefly from the papers of Weinberg and Séguin,[1] Hilda Hempel Heller,[2] Muriel Robertson,[3] and Karl Meyer.[4] It refers particularly to a saccharolytic non-proteolytic organism. This description also accords with the description given by Ghon and Sachs for their No. 11.

**Morphology.**—Bacilli with rounded ends measuring 0.6 to 0.8 $\mu$ in breadth by 3 to 5 $\mu$ in length in serous exudates and in young cultures. The ends are more rounded than in *Bacillus Welchii* and the organisms are thinner. It is usually in pairs, but may occur in threes and fours. In older cultures long forms, filaments, and degenerated elements are common. In the peritoneal exudate of infected animals the organism occurs usually in the classic long filaments made up of unequal elements. Kahn[5] states that the organisms measure 0.3 to 0.4 $\mu$ in width and 1.5 to 7 $\mu$ in length, average length 2.5 $\mu$. Deeply stained bulb-like forms occur especially in young cultures (M. R. C.[6]). In fluid media containing fresh

Fig. 157.—*Vibrion septique*, vegetative rods from dextrose agar stab three days old, stained with gentian-violet.

tissue, and on coagulated serum one sees great variation in morphology. Pale citron or navicular bodies occur with deeper staining points at one or both extremities, together with club-shaped forms, filaments, and bulbous organisms. Navicular forms occur also in tissues. Involution forms common on glucose agar (Adamson).

**Motility.**—*Vibrion septique* exhibits a great variation in motility. The organisms are either motionless or show a peculiar undulatory motility in serous exudates. In cultures the motility is less than in exudates. The presence of glucose and acid seems to hinder the motility while protein substances render it more active. Flagella are easily demonstrated by Loeffler's and by Zettnow's methods. They vary in number from 4 to 16, are peritrichic in arrangement, often consisting of bunches of stiff spirals. Occasionally there are long giant cilia, especially on glucose gelatin. Motility occurs only in the absence of atmospheric oxygen.

**Staining Reactions.**—*Vibrion septique* stains easily with the usual anilin dyes and retains Gram's stain in young cultures and in the tissues. In old cultures the organism gradually becomes Gram-negative. The absolute alcohol should not be allowed to act too long, otherwise the organisms decolorize.

**Capsules** not present.

**Spores.**—Spore-formation takes place readily in the tissues of man and infected animals, especially in the muscles. The organisms enlarge and the spores arise usually in the centers, less frequently subterminally. In cultures the spores form early, especially in cultures rich in protein and poor in carbohydrate, like muscle broth. In broth with brain substance or muscle tissue and in plain broth with white of egg sporulation is abundant. It is poor in milk. In glucose broth it occurs best on the third to fifth day. Sporangia look like short-handled tennis rackets (Kahn).

Spores large, oval, 1.5 by 1 $\mu$ (Kahn). Sporulation abundant in brain media (Hall[7]). Immature spores form the so-called orgonts of Heller.

**Cultural Characters.**—*Agar colonies* regular, entire, no radiating processes, small, translucent, moist (Adamson[8]).

The Medical Research Committee reports the agar surface colonies as fairly opalescent, contours smooth or indented, edges more or less crenated. Deep agar colonies show delicate branching, centers never compact or well-defined.

Hall describes the deep colonies as semitransparent with filamentous or wooly outgrowths.

*Agar Slant.*—Translucent or semitransparent film.

*Agar Stab.*—Growth along line of inoculation with few bubbles of gas.

*Deep Glucose Nitrate Agar* of Veillon.—Rapid development of colonies in the lower part of the tube. Colonies flocculent, arborescent, like those originally described by Roux.[9] Occasionally the colonies are lenticular and heart-shaped or show an extrusion of hair-like filaments from the sides or poles. The medium is acidified and there is an abundant gas production, not, however, so marked as in cultures of *Bacillus Welchii.*

*Glucose agar surface colonies* small, regular, semitransparent (Adamson).

*Glucose Gelatin.*—Fine, delicate colonies somewhat softer than in agar. Gelatin slowly liquefied.

*Gelatin Stab.*—Some gas production and liquefaction. Medium clears, pH 6.4 after eight months (Kahn). Kendall, Day, and Walker[10] find that little ammonia or aminonitrogen accumulates. No soluble gelatinose.

*Coagulated Serum Stab.*—Rapid development with an acid reaction. Serum not liquefied.

*Inspissated Serum.*—Gas production and separation of clot (Kahn). No digestion. Adamson describes small, moist, colorless colonies on inspissated serum. Serum becomes opaque and is not liquefied.

*Muscle Broth* (Weinberg and Séguin).—Abundant growth with the odor of butyric acid. The muscle-fibers are swollen, reddish, dissociated, but not digested.

*Cooked Meat.*—Gas production. Meat turns copper-red. Fluid clears. No reduction in total size of meat particles. Final pH after eight months = 6.4. Wolfe[11] reports gas, volatile acid, and ammonia in cooked meat, little amino-acid. No blackening even when metallic iron present. Odor not rancid (M. R. C.). No $H_2S$.

*Dorset's Egg Medium.*—Small, circular, colorless surface colonies (Adamson). No digestion, liquefaction, or blackening. When colonies are confluent they give a moist film.

*Cooked Codfish Medium* (Kahn).—Small amount of gas. No other reaction.

*Milk.*—In fresh milk a rapid production of acid and gas with an early coagulation. In old milk the coagulation appears later. The clot is at first massive, later it contracts somewhat, and is infiltrated with fine gas bubbles. Wolfe states that there is as much gas as with *Bacillus Welchii*, but that it appears more slowly. It consists of carbon dioxid and hydrogen, $\frac{CO_2}{H_2} = \frac{40.8}{59.2}$. Lactose is used up. Volatile acids produced. Ammonia increased markedly. More proteolysis than with *Bacillus Welchii*. Kendall, Day, and Walker report only minimal nitrogenous changes in milk. Hall reports no action on milk except with blood, when there may be gas production and coagulation of the casein.

*Litmus Milk.*—Rapid decolorization followed by coagulation. Clot white, not digested.

*Bromcresol-purple Milk* (Kahn).—Firm acid clot with gas. Clot breaks up and separates. Fluid clear, pH 5.8 after eight months.

*Martin's 0.2 Per Cent. Glucose Broth.*—Rapid development of turbidity with gas and acid. The organisms are precipitated to the bottom of the tube in twenty-four to forty-eight hours, forming a deposit which is often 1 cm. or more in height. Odor of buytric acid, neither repulsive nor putrid.

*Glucose Peptone.*—More gas and volatile acid than with ordinary peptone (Wolfe). Ammonia and amino-acid the same as with ordinary peptone.

*Martin's Broth without Glucose.*—Growth poor and scanty.

*Tryptic Broth* (Wolfe).—Gas production. No change in volatile acids. Amino-acids produced.

*Tryptic broth* containing a piece of rabbit's kidney. Gas, ammonia, and volatile acids higher than in plain tryptic broth. Amino-acid increased. Gas, carbon dioxid, and hydrogen, $\frac{CO_2}{H_2} = \frac{52.7}{47.3}$.

*Blood Broth.*—Rapid growth with solution of the corpuscles, the laked blood assuming a brownish color. The medium develops a strong butyric acid odor. Rapid sporulation.

*Egg Broth* of Besredka and Jupille.—Abundant development with moderate acidity. The egg-albumin is progressively and completely precipitated to the bottom of the tube with a clear, transparent, limpid liquid above.

*Martin's Broth without Glucose Containing Coagulated White of Egg.*—No attack on the coagulated egg-white even after a month.

*Solidified Egg Albumin Cube Broth* (Kahn).—Gas. Fluid cloudy, then clears. No proteolysis, pH 6.6 after 8 mo.

*Robertson's Alkaline Egg.*—Gas. Soft, mushy clot. No separation or digestion, pH 6.5 after 8 mo.

*Modified Egg Water* (Kahn).—Soft mushy clot. Gas. Clot becomes firm and tough and torn by gas, shrunken. Clear, watery fluid. No digestion.

*Brain Broth.*—Rapid development with an acid reaction. No blackening.

**Saccharolytic Properties Marked.**—Active fermentation of glucose, lactose, and maltose (Weinberg and Séguin). According to the Medical Research Committee and to Hall this organism ferments glucose, levulose, galactose, maltose, lactose, and salicin, but not glycerin, saccharose, inulin, mannite, or dulcite. Kendall, Day, and Walker report no fermentation of starch, and a fermentation of saccharose in some strains, failure to ferment in other strains, making two types. Kahn says *Vibrion septique* ferments glucose, levulose, galactose, saccharose, maltose, lactose, raffinose, xylose, starch, inulin, salicin, and glycerin. Many authors separate *Vibrion septique* from *Bacillus chauvei* by the non-fermentations of saccharose and the fermentation of salicin. Recently Scott has pointed out that this organism ferments arabinose, dextrin, dulcitol, galactose, glucose, glycerol, inulin, lactose, levulose, maltose, mannitol, mannose, raffinose, rhamnose, sucrose, salicin, starch, and xylose. The action in rhamnose, salicin, starch, and xylose serve to differentiate it from *Bacillus chauvei.*

**Proteolytic properties** very limited. Gelatin softened, but no true proteolysis occurs. No digestion of egg-albumin, casein, coagulated serum, brain or meat, and no blackening even when metallic iron present. No $H_2S$. No indol.

Decolorization of neutral red, litmus, methylene-blue, and indigo sulphate of sodium (Meyer).

**Pathogenic Properties.**—Vibrion septique is highly virulent for laboratory animals, especially for guinea-pigs, mice, white and gray rats, and pigeons, less so for rabbits. Meyer states that the rabbit is also sensitive. Nicolle, Césari, and Raphaël[12] have found that 1/10 to 1/30 c.c. of a 0.2 per cent. glucose culture given subcutaneously will kill guinea-pigs in twenty-four to thirty-six hours, while 1/50 c.c. is rarely fatal. Subcutaneous injection produces an emphysematous edema like that produced by *Bacillus Welchii* and *Bacillus chauvei* (Hall). The affected area is hot, moist, crepitant, and tender to the touch. The hair slips off easily. The organisms are present in the heart's blood. Intramuscular injection is less apt to be fatal. With rabbits, however, intramuscular injection is more apt to be fatal than subcutaneous, 1/10 to 1 c.c. killing the animals in twenty-four hours. Mice are very sensitive, according to Weinberg and Séguin, one of their strains after five passages through mice killing them by intramuscular injections of 1/1000 c.c.

**Toxins.**—In the opinion of Nicolle, Césari and Raphaël, Leclainche and Morel,[13] and Jouan[14] *Vibrion septique* secretes a soluble toxin which kills animals rapidly by intravenous inoculation with paralysis and convulsions. Subcutaneously the toxin produces extensive necrosis leading to the formation of a moist slough.

**Hemotoxins.**—Filtrates of cultures dissolve the blood-corpuscles of guinea-pigs, rabbit, sheep, horse, and man. In young cultures (sixteen hours) in Martin's 0.2 per cent. glucose broth 1/10 c.c. of the whole culture gives complete hemolysis of the corpuscles of man, sheep, and guinea-pigs. Old cultures are less hemotoxic.

**Hemagglutinins.**—Nicolle, Césari, and Raphaël state that the corpuscles of rabbits and guinea-pigs are agglutinated by filtrates of *Vibrion septique.*

**Antitoxins.**—Weinberg and Séguin have immunized a horse to the toxin of *Vibrion septique* and have obtained a serum which neutralized whole cultures and filtrates. Relative small quantities, $\frac{1}{10}$ c.c. of the culture, should be employed in testing. This represents 10 to 100 fatal doses for mice. Larger amounts of cultures, $\frac{1}{4}$ c.c., are not completely neutralized by large doses of antitoxin.

**Agglutinating Sera.**—Sera agglutinating *Vibrion septique* have been prepared by Leclainche and Vallée,[15] Markoff,[16] and Meyer. For homologous strains the sera agglutinate the organisms in high dilutions, 1 : 10,000 and 1 : 20,000. Such sera do not agglutinate closely related organisms like *Bacillus chauvei* to anything like the same degree.

1. Weinberg and Séguin: La gangrène gazeuse, 1916, p. 79.
2. Heller: J. Infect. Dis., 1920, xxvii, 385.
———: J. Bact., 1922, vii, 1.
3. Robertson: J. Path. and Bacteriol., 1916, xx, 327.
4. Meyer: J. Infect. Dis., 1915, xvii, 458.

5. Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.
6. Rep. Brit. Med. Research Com., No. 39.
7. Hall: J. Infect. Dis., 1922, xxx, 445.
8. Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.
9. Roux: Ann. d. l'Inst. Pasteur, 1887, i, 49.
10. Kendall, Day, and Walker: J. Infect. Dis., 1922, xxx, 141.
11. Wolfe: J. Path. and Bacteriol., 1918–19, xxii, 115.
12. Nicolle, Césari, and Raphaël: Ann. de l'Inst. Pasteur, 1916, xxix, 165.
13. Leclainche and Morel: Ann. de l'Inst. Pasteur, 1901, xv, i.
14. Jouan: Cited by Nicolle, Césari, and Raphaël.
15. Leclainche and Vallée: Ann. de l'Inst. Pasteur, 1900, xiv, 595.
16. Markoff: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1911, lx, 188.

### Gram-negative strains of Clostridium septicum

From time to time strains of *Vibrion septique* or of *Bacillus œdematis-maligni* have been isolated which have been described as Gram-negative. Allowing for all differences in technic it is evident that certain strains of these organisms retain Gram's stain very feebly or not at all. Such strains have been described by Eisenberg,[1] by Muir and Ritchie,[2] Eugene Fraenkel,[3] Ghon and Sachs.[4]

1. Eisenberg: Przeglad eekarski. 1889, pp. 606 and 625.
2. Muir and Ritchie: Manual of Bacteriology. 1910, p. 433.
3. Fraenkel: Beitrag zur Klinik der Infektionskrankheiten, 1915–16, p. 129.
———: München. med. Wchnschr., 1916, lxiii, 1, 476.
4. Ghon and Sachs: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1903, xxxiv, Orig., 289.

### Clostridium œdematis-maligni (Zopf)

The organism usually known as the malignant edema bacillus was observed by Koch[1] who regarded it as the same as Pasteur's *Vibrion septique.* It was first brought to cultivation by Gaffky.[2] Named *Bacillus œdematis-maligni* by Zopf. Ever since that time a spirited controversy has existed as to whether Koch's bacillus is identical or not with *Vibrion septique* and whether it will produce the clinical picture of malignant edema. The usual opinion at present is that Pasteur's organism, which fortunately has been kept alive, is essentially a pathogenic saccharolytic anaërobe and that the organism described by Koch is essentially a proteolytic organism possessed of feeble if any pathogenic powers and resembling or identical with the organism described later by Metchnikoff as *Bacillus sporogenes.* It may be noted that the condition known as malignant edema, which is wide-spread in animals and occurs but rarely in man, is by no means a clearly defined clinical entity. Further, the contention of Henry and others that anaërobes owe their pathogenic powers to an ability to act upon sugar (muscle sugar, glycogen, etc.), is by no means established. It is entirely possible that a proteolytic anaërobe may be pathogenic. Furthermore, cases of so-called malignant edema have repeatedly shown the presence of organisms like Koch's malignant edema bacillus. The question can hardly be regarded as settled as yet, and for the present Koch's bacillus of malignant edema should be regarded as a distinct species. We follow the description given by v. Werdt[3] and by von Hibler[4] for his No. X. The proteolytic malignant edema bacillus, Ghon and Sachs No. II, is probably the same organism.[5]

**Morphology.**—Rather small rods with rounded ends measuring 1 $\mu$ in thickness by 3 $\mu$ in length. They show great variations in size, forming long threads in serous exudates and in cultures. In media containing dextrose or maltose large bladder forms are rarely present, showing granular contents. Von Hibler found such forms best in alkaline media. They are of various shapes—round, spherical. pear-shaped, etc., and stain bright yellow with iodin potassium iodid.

**Staining Reactions.**—The organisms stain by the usual dyes and retain Gram's stain provided that they are not treated with alcohol too long.

**Motility.**—Progressive motility both in the individual bacilli and in the long threads (von Hibler). Motility can best be brought out by cultivation in brain broth. The sporulating rods are likewise motile. Flagella can be stained by the usual methods. They number from 12 to 20, arranged at the poles and about the bodies of the organisms (peritrichic).

**Spore-formation.**—Spores are formed readily both in cultures and in infected tissues. They are formed in neutral and alkaline media and abundantly in milk and in brain broth. The spores are oval or ellipsoidal, usually wider than the bacilli, and are generally formed in the middle of the organism, giving it the appearance of a clostridium. They may also form at the ends of the rods.

**Resistance.**—The spores in brain broth are very resistant to heat, withstanding the action of streaming steam two and one-half hours.

**Methods of Cultivation.**—The malignant edema bacillus is a strict anaërobe developing in a vacuum, in an atmosphere of carbon dioxid, of hydrogen, or in the bottom of deep agar tubes.

*Deep agar colonies* are irregular clumps showing on the edges prolongations of fine hairy processes. Under low power they are networks of fine threads. These colonies are like those of Novy's bacillus, Ghon and Sachs bacillus (*Vibrion septique*), but differ from colonies of *Bacillus Welchii* or *Bacillus chauvei*.

*Surface* agar colonies under anaërobic conditions are like the deep colonies but with darker nuclei and circumferences made up of fine filamentous prolongations. Von Hibler finds that the colonies are occasionally round, regular, with entire edges without processes.

*Deep gelatin colonies* are spherical with radial striations. Surface colonies show the same type of structure.

*Gelatin Stab.*—Development along line of inoculation as a white streak with lateral branching. The gelatin is liquefied and a white sediment collects at the bottom.

*Brain Broth.*—Rapid development with an alkaline reaction which blackens the particles of brain substance through the formation of hydrogen sulphid. There is considerable gas production and a foul odor.

*Milk.*—Gradual clotting and peptonization. This takes place in an ordinary atmosphere.

*Coagulated Blood-serum.*—Rapid development of the organisms with digestion of the serum. If the reaction is very alkaline, the liquefaction does not occur unless the alkali is neutralized with lactic acid. The serum is gradually blackened. Fibrin, blood, and liver tissue are digested in the same way as blood-serum. Muscle is digested and turned black, with a foul odor.

*Carbohydrates* are not fermented, but evolve a foul odor. The organisms grow on potato under anaërobic conditions without gas formation.

**Pathogenic Action.**—White and gray rats, guinea-pigs, and rabbits are susceptible to experimental inoculation (von Hibler). Following subcutaneous injection the animals develop spreading local edema and die in twenty-four hours. At autopsy there is an intense hemorrhagic edema with more or less abundant gas development and but little or no necrosis of tissue. Microscopically small, necrotic areas are found in the muscular tissue which shows a degeneration of the muscle fibers.

**Differentiation from Other Anaërobes.**—Von Hibler believes that the true malignant edema bacillus can be differentiated from the Ghon and Sachs bacillus (*Vibrion septique*) by its pathogenic action. The former produces a bloody edema, the Ghon and Sachs bacillus a serous edema. Culturally it digests striated muscle, liquefies coagulated blood-serum, blackens brain broth, and peptonizes casein, all of which reactions are not given by the Ghon and Sachs bacillus. It can also be distinguished from Novy's bacillus and from Liborius's pseudo-edema bacillus.

1. Koch: Mitt. a. d. K. Gsndhtsamte., 1881, i, 49.
2. Gaffky: Mitt. a. d. K. Gsndhtsamte., 1881, i, 80.
3. v. Werdt: *In:* Handbuch. d. path. Mikroorgan. (Kolle and Wassermann). 2. Aufl. 1912, iv, 837.
4. von Hibler: Untersuchungen über die pathogenen Anaëroben. Jena, 1908.
5. Ghon and Sachs: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1909, xlviii, Orig., 396.

### CLOSTRIDIUM CHAUVEI (Arloing, Cornèvin, and Thomas)

*Bacillus chauvei*, known also as *Bacillus anthracis symptomatici*, was first observed by Arloing, Cornèvin, and Thomas[1] in the condition called Quarter-evil, Symptomatic anthrax, Blackleg, or Rauschbrand. This is an ill-defined clinical entity and may be due to a variety of anaërobes. The most frequently isolated organism is a motile anaërobe resembling *Vibrion septique*

Fig. 158.—*Bacillus chauvei* from twenty-four-hour culture in dextrose agar, stained by Gram.

Fig. 159.—*Bacillus chauvei* from three-day culture in dextrose agar, stained by Gram.

of Pasteur. It was first brought to cultivation by Kitasato. Not found definitely in man.

**Morphology.**—Large, rod-shaped organisms measuring 0.6 to 1 $\mu$ in diameter by 2.5 $\mu$ in length, occurring singly and in pairs, practically never in chains. Navicular and club-shaped forms occur in serum media or in the presence of fresh tissue. They are longer and more slender than the similar forms found in *Vibrion septique*. They are also present in infected guinea-pigs. Such solidly stained, swollen forms are called orgonts by Heller.[2] *Bacillus chauvei* does not form filaments on the peritoneal surface of the liver in experimental animals and may thus be differentiated from *Vibrion septique* (Leclainche and Vallée,[3] Meyer,[4] Robertson[6]).

**Motility.**—Active from peritrichic flagella.

**Spores** formed readily. They lie in the center of the rods or toward one end, are wider than the rod to which they impart the clostridial form. They are oval or elliptical. They are not formed in the tissues of infected animals during life, but appear rapidly after death, in twenty-four to forty-eight hours.

**Stained** easily with the usual anilin dyes. Gram-positive in young cultures. Rapidly becomes Gram-negative.

**Resistance.**—The spores are very resistant to drying, heat, and antiseptics (Jordan). Not killed by half an hour's exposure to 100° C.

**Cultural Reactions.**—*Bacillus chauvei* is a strict anaërobe which grows poorly on artificial media, especially when first isolated. Later the organism grows well when blood or blood-serum is added to the media.

*Agar Colonies.*—Somewhat filamentous or with granular compact centers and thin peripheral zones. Under low power the colonies show a mass of fine tangled threads. Blood-agar surface colonies flat, leaf-like, or round. Actively hemolytic. Deep colonies semi-transparent, opaque spheres with or without ray-like growth (Hall[6]). Liver agar (2 per cent.) colonies small, spherical, or pin-point (Scott).

*Agar Stab.*—Abundant growth along line of inoculation with the production of considerable amounts of gas which break up the agar into small bits.

*Gelatin Plates.*—Round or oval colonies with compact centers and fine radiating peripheral filaments.

*Gelatin Stab.*—Growth abundant in the depths of the medium, consisting of rounded, lobulated colonies with a dark center and clear outer zone. The gelatin is liquefied. Scott reports absence of liquefaction in plain gelatin, liquefaction of glucose gelatin.

*Milk* shows little change (Hall). With sterile blood added there may be gas production and coagulation of the casein. Casein clot not digested. The Medical Research Committee[7] state that milk shows acid and gas production, clotting in three to six days.

*Broth* containing blood or blood-serum gives an abundant growth with the evolution of much gas. This gas is without odor (Kitt[8]).

*Dextrose Broth.*—Abundant development with the evolution of a gas made up of carbon dioxid, hydrogen, and methane in traces, with traces of butyric and lactic acid. Scott[9] finds that *Bacillus chauvei* ferments glucose, galactose, glycerol, inulin, lactose, levulose, maltose, mannitol, and sucrose, rarely arabinose, dextrin, mannose, and raffinose, but not rhamnose, salicin, starch, and xylose.

*Chopped Meat.*—Gas production. Pink color fading. No blackening. Non-putrid.

*Brain Medium.*—No digestion or blackening.

*Coagulated Serum.*—Not liquefied.

**Pathogenic Action.**—The organism is virulent for guinea-pigs, cattle, sheep, and goats, but not usually for rabbits, dogs, cats, or birds. Man is immune to the natural disease. A rapidly spreading edematous lesion is produced with a falling out of the hair and a blackening of the skin. On opening the body an intense edema infiltrated with gas bubbles is found. Washed spores are not pathogenic according to Leclainche and Vallée.

**Toxin.**—Leclainche and Vallée[10] state that a soluble toxin is produced in Martin's broth which is quite resistant to heat, but rapidly deteriorates in the air. Scott has been unable to obtain such a toxin.

**Vaccines.**—Blackleg vaccines are widely manufactured and used commercially. Two vaccines are employed. Number 1 is made from the dried juice of infected material rubbed up in water and heated to 100° C. for six hours. Vaccine No. 2 is the same material heated to 94° C. for four hours. Kitt uses the whole meat, dried and powdered and heated to 94° C. for six hours. Animals were first successfully immunized to cultures by Arloing, Cornèvin, and Thomas. Nishiura[11] has recently immunized animals with diluted cultures and with filtrates.

*Bacillus chauvei* is differentiated with some difficulty from *Vibrion septique*. It is highly saccharolytic, but feebly proteolytic, attacking gelatin only, thus agreeing with the French conception of Pasteur's organism. According to Muriel Robertson it ferments saccharose and not salicin, while *Vibrion septique* ferments salicin and not saccharose. This

has been confirmed by Scott, who finds that rhamnose, salicin, starch, and xylose are not attacked by *Bacillus chauvei* and are acted on by *Vibrion septique*. *Vibrion septique* is more virulent, grows more rapidly than *Bacillus chauvei*, and is much more strongly Gram-positive. It tends to produce long snake-like filaments in infected animals.

Both Meyer and Weinberg and Séguin[12] have prepared sera from clearly identified strains of *Vibrion septique* which agglutinate this organism in high dilution (1 : 20,000 Meyer) (1 : 1000 Weinberg and Séguin), but do not agglutinate *Bacillus chauvei* beyond a dilution of 1 : 100 (Meyer) or 1 : 10 (Weinberg and Séguin).

1. Arloing, Cornèvin, and Thomas: Le charbon symptomatique de boeuf. Paris, 1887.
2. Heller: J. Infect. Dis., 1920, xxvii, 385.
3. Leclainche and Vallée: Ann. de l'Inst., Pasteur, 1900, xiv, 595.
4. Meyer: J. Infect. Dis., 1915, xvii, 458.
5. Robertson: Brit. M. J., 1918, i, 583.
6. Hall: J. Infect. Dis., 1922, xxx, 445.
7. Rep. Brit. Med. Research Com. No. 39.
8. Kitt: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 1903, ii, p. 601.
9. Scott: J. Bact., 1925, x, 266.
10. Leclainche and Vallée: Ann. de l'Inst., Pasteur, 1909, xiv, 595.
11. Nishiura: Centralbl. f. Bakteriol. [etc.], 1924, xci, 401.
12. Weinberg and Séguin: La gangrène gazeuse. 1916.

### Clostridium of whale septicemia (Neilson)

Originally found by Nielson[1] in a septicemia of whales due to wound infection and regarded as similar to but not identical with B. *chauvei*. Slightly different organisms were also observed from infected whale muscle by Christiansen.[2] Recently Hilda Hempel Heller[3] has obtained an organism closely resembling *Vibrion septique* from infected whale muscle. Probably several different types of anaërobes have been encountered in and are capable of giving rise to wound infections of whales.

1. Nielson: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1890, vii, Orig., 207.
2. Christiansen: Norsk Mag. f. Lægevidensk, 1919.
3. Heller, Hilda Hempel: J. Infect. Dis., 1920, xxvii, 385.

### Clostridium Novyi (Migula)

First described from infected guinea-pigs by Novy[1] as *Bacillus œdematis maligni* No. II. Organisms subsequently isolated by Kerry[2] from blackleg in cattle were identified by him as Novy's bacillus. Culturally similar to *Bacillus œdematiens* of Weinberg and Séguin who regard it as the same. Apparently the same as v. Hibler's No. 5. Novy's description is as follows:

**Morphology.**—In infected tissues a straight thin bacillus with rounded ends, measuring 0.8 to 0.9 $\mu$ in width by 2.5 to 5 $\mu$ in length. Generally 3.5 to 4.3 $\mu$ in length. The organisms usually lie singly and may form threads 8 to 14 $\mu$ long, rarely 22 to 35 $\mu$ long. In artificial cultures the organisms show a great variety of forms. In deep agar stabs they appear as bent rods and short threads. On the surface of agar they are curved, even comma-shaped, and also form long threads. In broth the bacilli show many small round refractile bodies, either free or lying at the ends of the organism.

**Motility.**—Moderate whirling motility much less than that of *Bacillus chauvei* or of Koch's malignant edema bacillus. The flagella are easily stained by Loeffler's method when acids or alkalies are added to the mordant. The flagella are long, wavy, and attached to the sides and ends of the organism. Giant flagella are common, spindle shaped, tapering to barely visible ends. They vary in length from 40 to 50 $\mu$ and may be 72 or more $\mu$ long. These giant flagella may be observed in unstained preparations. They are formed best in gelatin and agar cultures.

**Spores.**—Not observed by Novy, whose cultures nevertheless withstood temperatures of 58° C. for an hour.

**Staining Reactions.**—The organism stains well with the usual anilin dyes and is Gram-positive.

**Cultural Reactions.**—Novy's bacillus is an obligate anaërobe cultivated with some difficulty. The best media for this purpose are weakly alkaline broth, gelatin, or agar with 2 per cent. peptone and 2 per cent. grape-sugar. It does not develop below 24° C. and grows

best at about 35° to 38° C. It develops in a vacuum or in an atmosphere of hydrogen, carbon dioxid, nitrogen, or even illuminating gas. A complete vacuum is not essential. A reduction of the pressure to 60 to 65 mm. mercury suffices to permit development.

*Glucose Agar Colonies.*—The organism develops rapidly, forming small white colonies the size of the head of a needle in fifteen hours. Under the microscope they show a thick tangle of threads not unlike those of *Bacillus tetani*. The larger colonies show dark centers surrounded by zones of fine threads. Under low power small fine black granules may be present on the ends of the threads.

*Glucose Agar Stab.*—In the ordinary atmosphere an abundant development along the line of inoculation in the depths of the agar, extending almost but not quite to the upper surface. At 38° C. the organisms develop in twelve to sixteen hours. After twenty-four hours the growth ceases. Abundant evolution of gas which breaks the agar to pieces in alkaline, but not in acid or neutral media. Young cultures give off a strong odor of butyric acid. In an atmosphere of hydrogen the surface of the agar may be covered with a thin white membrane and the water of condensation becomes turbid.

*Glucose Agar Slant.*—In an atmosphere of hydrogen the isolated colonies are round, white, somewhat elevated. They combine to form a clear white membrane. Condensation water turbid.

*Gelatin.*—Little development at 25° C. With 10 to 15 per cent. gelatin containing 2 per cent. glucose with or without litmus the organisms grow well in the thermostat, producing a turbidity and marked gas formation. The lower part of the gelatin becomes quite turbid. On transfer to room temperature the gelatin is solidified completely, so there is no liquefaction.

*Broth.*—Under anaërobic conditions the organisms cause a uniform turbidity. The addition of 2 per cent. peptone and 2 per cent. glucose favors the growth of the organisms, the broth becomes acid and considerable gas develops. The addition of sodium indigo sulphuric acid has no effect on the growth of the organisms, but the substance is reduced. Litmus is likewise reduced to a colorless leuko-compound.

**Pathogenic Action.**—Novy's bacillus is virulent for guinea-pigs, white rats, white mice, rabbits, cats, and doves. Small doses, $\frac{1}{10}$ to $\frac{1}{4}$ c.c., suffice to kill the animals in twelve to thirty-six hours. At autopsy an abundant colorless brawny edema is found at the site of subcutaneous inoculation, spreading to adjacent areas. Occasionally the edematous material is light red in color and there are small quantities of gas present.

Hall[3] reports that Novy's original, a strain of *Bacillus œdematiens* of Weinberg, and an isolation of his own in California are identical. These strains differ from Novy's original description in certain particulars. They produce rather large subterminal spores, hemolytic colonies on blood agar and liquefy and blacken gelatin. They have no visible action on milk. They cause no blackening or digestion of coagulated albumin, meat, brain or egg-white. Little or no $H_2S$ produced. Fermentation occurred in glucose and glycerol, but not in lactose, saccharose, salicin, and inulin.

Henry[4] finds a fermentation of glucose, levulose, maltose, xylose, and starch. A recent report by Kendall, Day, and Walker[5] states that glycerol, galactose, saccharose, lactose, mannite, dulcite, inulin, and salicin are not fermented.

1. Novy: Ztschr. f. Hyg., 1894, xvii, 209.
2. Kerry: Oester-Ztschr. f. wissensch. Veterinärk., 1894, v.
———: Centralbl. b. Bakteriol. [etc.], 1. Abt., 1894, xvi, Orig., 372.
3. Hall: J. Infect. Dis., 1922, xxx, 445.
4. Henry: J. Path. and Bacteriol., 1916, xxi, 344; 1920, xxiii, 254.
5. Kendall, Day, and Walker: J. Infect. Dis., 1922, xxx, 141.

### Clostridium of Bradsot (Neilson)

An obligate anaërobe found in the disease called variously braasot, bradsot or braxy, an acute infection of sheep. First isolated and described by Ivar Neilson.[1] The etiology of the disease is by no means cleared up, but Jensen[2] regards this organism as the cause of the infection. The disease is wide-spread in Norway, Scotland, the Shetland Islands and also in Tasmania and New Zealand.

**Morphology.**—Large bacillus with rounded ends measuring 1 $\mu$ broad by 2 to 6 $\mu$ long. It occurs usually singly but chains and long threads may be found in serous exudates and in the internal organs of infected animals. Involution forms, swollen and lemon-shaped, are present occasionally in old cultures.

**Spore-formation.**—Spores are formed readily both in the animal body and in cultures. The spores are large and oval, lying in the middle of the rods which are swollen. Sometimes

the spores are on the ends of the rods. They are easily killed by boiling in moist conditions. When dried they resist a temperature of 100° C. for several hours.

**Motility.**—Positive motility from peritrichic flagella. These vary in number, usually 12 to 20 being present, long, thin, and wavy. The flagella are stained with some difficulty.

**Staining Reactions.**—Gram-positive.

**Cultural Reactions.**—The Brodsot bacillus is an obligate anaërobe cultivated without great difficulty on the usual media in a vacuum and in an atmosphere of nitrogen or hydrogen. Development is favored by the addition of sugar. It develops at ordinary temperatures and especially between 35° and 72° C.

*Agar Colonies.*—Under anaërobic conditions the deep colonies appear in twenty-four hours as biconvex bodies with smooth edges and yellow-brown or dark yellow granular contents. These colonies rapidly send out branches or threads so that they assume a feathery, felty appearance. Superficial colonies occasionally develop as cloudy white specks.

*Gelatin Colonies.*—Development in gelatin is somewhat slower than in agar. After several days round, cloudy colonies appear consisting of liquefied gelatin. These increase in size and show superficial radial striations under low magnification.

*Serum Agar Plates.*—In mixtures containing 3 parts of agar and 1 part of serum the colonies in twenty-four hours at 37° C. are small, about the size of millet seeds, and white with bushy, feathery surfaces. In equal parts of agar and blood-serum the colonies in twenty hours are much larger, measuring ½ to 1 cm. They coalesce rapidly and become turbid and cloudy. Abundant development of gas bubbles.

*Serum Agar Stab.*—With small proportions of serum to agar there is rather scanty development. With larger quantities of serum the medium rapidly becomes turbid, with an abundant development of foul-smelling gas.

*Serum Broth.*—An abundant development with evolution of much gas with a foul odor. The serum constituents of the mixture are digested to a turbid jelly-like mass of clumps.

*Coagulated Serum.*—Abundant development with evolution of foul-smelling gas. The serum is digested and gradually becomes turbid.

*Brain Broth.*—Abundant development with the production of an acid reaction. No digestion of brain substance.

*Milk.*—Acid production and rapid coagulation. No digestion of the casein.

**Fermentative Properties.**—The bradsot bacillus ferments dextrose, mannose, galactose, fructose, lactose, maltose, glycerin, but does not act on saccharose, raffinose, sorbose, arabinose, xylose, rhamnose, mannite, dulcite, adonite, and erythrite (Bahr[3]).

**Reaction of Media.**—The organism develops little or none in acid media, but abundantly in alkaline media. With sugar media development ceases after the media become clearly acid.

**Pathogenic Action.**—Jensen states that subcutaneous inoculation of the cultures in sheep produces lesions like those in symptomatic anthrax. There is a blood-stained, serous exudate in the subcutaneous tissues with an abundant evolution of gas consisting presumably of hydrogen or methane. There is a hemorrhagic infiltration of the neighboring muscles with an abundant evolution of gas in them also. After the death of the animal the development of gas is very rapid and the cadaver rapidly becomes foul and stinking. A number of other animals such as calves, goats, pigs, guinea-pigs, pigeons, and chickens are sensitive to inoculation, while rabbits and mice are somewhat more resistant. Filtrates have little effect on animals except in very large doses. Jensen states that the organism exerts a negative chemotactic action on leukocytes. After thorough heating it shows positive chemotaxis. Similar observations have been made by Besson, Leclainche, and Vallée on malignant edema (*Vibrion septique*) and Rauschbrand.

**Remarks.**—From the description it seems that the bradsot bacillus could be distinguished with great difficulty from the organisms causing symptomatic anthrax or from *Vibrion septique*. According to McGowan[3] bradsot or braxy is the hyperacute form of hemorrhagic septicemia and is caused by *Bacillus bipolaris septicus*.

1. Ivar Neilson: Monatshr. f. prakt. Tierh., 1896, viii.
2. Jensen: *In:* Handb. d. path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, vi, 224.
3. McGowan: Centralbl. f. Bakteriol. [etc.], 1923, xci, 54.

### CLOSTRIDIUM BOTULINUM (van Ermengem)

Discovered by van Ermengem[1] in a peculiar form of meat poisoning known as botulism or allantiasis and isolated from the ham which caused the outbreak, from the spleen, and from the contents of the stomach and intestine in one of the fatal cases. In Europe the principle foods which have caused botulism are pig sausages, preserved meat, preparations of duck and geese, salted fish like mackerel (Madsen[2]) and salmon. In cases of clinical botulism

from vegetables (beans) organisms much like van Ermengem's bacillus have been isolated (Landmann, Gaffky[3]). These are known as the Darmstadt strain.

In America the cases of botulism have been traced chiefly to spoiled ripe olives, canned corn, and spinach. According to van Ermengem conditions which make for complete anaërobiasis in preserved meats, such as the growth of aërobic putrefactive bacteria, favor the growth of *Bacillus botulinus*. In many instances the foods which produce botulism are free from putrefactive organisms (N. Schmidt,[4] Nauwerck[5]) and have only the odor of butyric acid. The organism isolated by van Ermengem did not produce foul decomposition.

Fig. 160.—*Bacillus botulinus*, vegetative rods from three-day culture on dextrose agar, stained by Gram.

Clinically the cases of botulism present a great variety of symptoms, referable chiefly to the nervous system, with a high mortality. The disease is attributed to the absorption through the alimentary canal of poisons preformed in the foods and not to infection with the organism which apparently does not grow in the alimentary tract of man.

The following description applies to the van Ermengem strain[6]:

**Morphology.**—Large bacilli 4 to 6 $\mu$ long, 0.9 to 1.2 $\mu$ wide, with slightly rounded ends. They often appear in pairs end-to-end and frequently form long threads. Involution forms common in sugar-free media in which the growth is sparse.

**Motility.**—Moderate, due to fine peritrichic flagella 4 to 8 in number.

**Stained** by the usual anilin dyes. Gram-positive.

**Spores** formed in anaërobic conditions at temperature of 20° to 35° C. Spores oval or egg-shaped, usually terminal (subterminal ?), less frequently central so that the bacilli are clostridium or spindle-shaped. Spores not formed in sugar-free media.

**Cultivation.**—Cultivated only under anaërobic conditions and best isolated on media to which glucose is added.

*Glucose Gelatin Plates.*—Young colonies characteristic, round, transparent, pale yellow. Under low power they seem to be composed of large granules in active movements. The

colonies are surrounded by a narrow zone of liquefaction. Older colonies are brownish, opaque, and show a peripheral seam of fine thorn-like processes. Old colonies may be several millimeters in diameter.

*Glucose Gelatin Stab.*—Abundant development with the evolution of gas which breaks up the gelatin into small blocks. The gelatin is completely liquefied and often foamy. Large whitish flocculi are occasionally produced which sink to the bottom leaving the gelatin clear above.

No liquefaction of coagulated serum (M. R. C., No. 39).

*Deep Shake Glucose Agar Colonies.*—Semi-opaque, biconvex or kidney-shaped, 1 mm. in diameter in twenty-four to forty-eight hours (M. R. C., Report 39). They have a central nucleus and old colonies send out irregular projections. Considerable gas is produced.

*Glucose Agar Stab.*—Growth along line of inoculation with abundant evolution of gas which breaks up the agar into small bits. The expressed water of condensation becomes turbid.

*Glucose Broth.*—Diffuse turbidity with gas production gradually clearing. The gas evolved from glucose is hydrogen, carbon dioxid, and methane ($CH_4$). Moderate growth in saccharose and lactose broth. No fermentation.

*Milk.*—Slight growth without change in reaction.

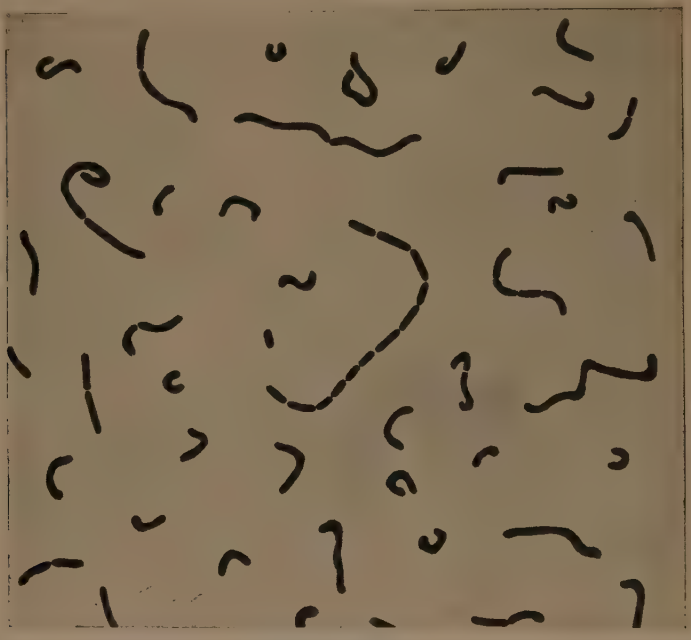

Fig. 161.—*Bacillus botulinus*, from eleven-day culture on glucose agar showing aberrant forms stained with Gram.

Abundant growth on van Ermengem's special medium consisting of equal parts of finely ground meat and water to which 2 per cent. glucose, 1 per cent. peptone, and 1 per cent. NaCl are added. The medium must be slightly alkaline and covered with a thick layer of fat or oil. With well-cooked media at 20° to 25° C. the fermentation lasts a week.

Little or no growth in agar, gelatin, broth or meat when sugar is not added. Non-sporulating, atoxic, and rapid dying out of the organism.

Good growth in sugar-free media made from calf liver or mashed mussels. Good development also on pure vegetable infusions such as green peas or potatoes to which peptone or salt is added. When 2 per cent. glucose is added an abundant fermentation occurs.

In all fermenting media a penetrating butyric acid odor is evolved.

*Bacillus botulinus* itself is an obligate anaërobe. In symbiosis with aërobic species, it grows under aërobic conditions as with white sarcinæ (van Ermengem), with *Bacillus subtilis* (Roemer[7]). It grows in the atmosphere in freshly prepared infusions of swine meat, calf liver, or vegetables. It also grows under aërobic conditions when small pieces of fresh guinea-pig kidney or liver, freshly cooked, finely divided animal tissues, or potatoes are added to broth tubes.

The optimum temperature for the strains of *Bacillus botulinus* isolated in Europe is 25° to 30° C., little growth occurring above this temperature.

The media should be slightly alkaline, but some strains grow on slightly acid media. Tchitchkine[8] and Leuchs[9] believe that the best medium for *Bacillus botulinus* is unneutralized swine meat infusion with 1 per cent. glucose, 0.5 per cent. NaCl and a small quantity of sodium carbonate added. Van Ermengem states that growth of *Bacillus botulinus* does not occur with more than 2 per cent. NaCl.

Spores less resistant than the spores of many anaërobes. Destroyed at 85° C. in fifteen minutes, at 80° C. in thirty minutes, by 5 per cent. phenol in twenty-four hours. In old cultures spores do not lose their viability even in the presence of considerable acid. In fused tubes protected from light and air cultures remain alive months and even more than a year.

Cultures should usually be transferred every six weeks.

The spores are widely distributed and have been isolated by Kempner[10] from the intestines of normal hogs.

**Pathogenic Action.**—*Bacillus botulinus* owes its pathogenicity entirely to a powerful toxin which is formed in various types of food and on a great many artificial media (van Ermengem[6]). Van Ermengem first obtained it in a medium of beef infusion broth containing 1 per cent. NaCl, 1 per cent. peptone, and 2 per cent. glucose. Different strains vary greatly in the amount of toxin which they produce. In rabbits a small quantity of glucose broth culture, 0.0003 to 0.001 c.c., suffices to produce a more or less general outspoken motor paralysis. This may develop suddenly or after three to four days and is quickly followed by the death of the animal. Sometimes only certain groups of muscles are affected so that the head falls to one side, the front legs are stretched out, the hind legs drawn up against the abdomen. In other instances the hind legs are paralyzed and the animal drags itself along with its front legs. At the same time there may be salivation, dilatation of the pupils, exophthalmos, suppression of urine and feces. Intoxication develops more slowly when the poison is introduced intraperitoneally. Injected intravenously in the ear vein the animals die in eighteen to twenty-four hours from 0.001 c.c. doses, while large quantities, 0.1 to 0.5 c.c., kill them in eight to fifteen hours from paralysis of the respiration. Intra-ocular injection produces dilatation of the pupils of both eyes. In guinea-pigs still smaller doses, 0.00005 to 0.0001 c.c., of glucose broth culture produce often a complete paralysis of all the muscles so that the animals lie motionless upon the abdomen with paws outstretched. Respirations are at first accelerated, then slowed. Attacks of dyspnea develop and the animals die in eighteen to thirty-six hours (of paralysis of the respiration). With smaller doses the animals develop chronic intoxication with paralysis of particular groups of muscles. Contractions of the muscles of the abdomen are frequently the first symptom of the disease. Larger doses are required to produce intoxication when introduced intraperitoneally. Mice are extremely sensitive, very minute doses producing fatal intoxication with paralysis of the hind limbs.

In apes (Rhesus) small doses, 0.001 to 0.1 c.c., produce a characteristic symptom-complex, dilatation of the pupils, ptosis of the lids, secretion of a thick grayish mucus from the nose and mouth, coughing and choking, aphagia, dyspnea, and death in twenty-four to thirty-six hours.

In cats the experimental intoxication is almost pathognomonic. Small subcutaneous doses are followed by marked dilatation of the pupils, complete relaxation of the third eyelid, aphonia, aphagia, prolapse of the tongue, secretion of a thick mucus from nose and mouth, coughing and suppression of urine and feces. After large doses, 5 to 10 c.c., the animals die in collapse and general paralysis in sixteen to twenty-four hours. With smaller doses, 0.1 to 1 c.c., death occurs after six to eight days and with still smaller doses, 0.01 c.c., the animal may recover or develop cachexia and die after weeks or months. White rats, dogs, hens, frogs, and fishes are almost completely resistant. Pigeons inoculated in the breast muscles with 0.5 to 1 c.c. quantities develop more or less complete paralysis of the muscles of the wings, ptosis, regurgitation of greenish fluid from the stomach. The feathers are ruffled and the birds motionless. Death occurs after several days.

*Feeding.*—Mice, guinea-pigs, and apes are easily poisoned by oral administration. Pieces of bread moistened with 1 to 2 drops of a fluid culture and fed to guinea-pigs produce outspoken paresis and death in twenty-four to thirty-six hours. Apes are poisoned by feeding small doses and die in twenty-four to thirty-six hours with the same symptoms as follow subcutaneous inoculation (van Ermengem). In rabbits large doses are required to produce fatal poisoning when introduced directly into the stomach. Cats, dogs, rats, and hens can eat large quantities of toxin and show only transient diarrhea and emaciation.

Botulismus toxin destroyed in three hours at 56° C., in half an hour at 80° C. (van Ermengem). It is highly toxic to man. Van Ermengem has reported the death of one individual from eating 200 grams of infected ham.

Antitoxins against the van Ermengem strain of *Bacillus botulinus* have been prepared by a number of European observers including Kempner,[10] Forssman and Lunstrom,[11] and Leuchs.[12] Their use has been disappointing.

The strain of *Bacillus botulinus* isolated by van Ermengem is no longer available for

study. According to Bengtson[13] the organisms described in the publications of the Medical Research Committee of Great Britain and known as Lister Institute Culture No 94 conform closely to the original description given by van Ermengem. It does not coagulate milk, does not digest casein, and does not liquefy coagulated egg-albumin and certain other proteins.

The Darmstadt strain described by Gaffky and Landmann differed in certain particulars from van Ermengem's original. It was more slender and less Gram-positive (Leuchs), grew readily and produced toxin at 37° C., and did not produce gas in glucose agar. Anaërobic cultures had an odor of Limburger cheese. The toxin secreted by the Darmstadt strain was not neutralized by the van Ermengem antitoxin and vice versa. Bengston states that the Lister Institute strain No. 95 resembles closely the Darmstadt strain, the original of which is no longer alive.

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**American Work in Botulism.**—Botulism was first observed in America by Nevin[1] who isolated *Bacillus botulinus* from cottage cheese which had caused three fatal cases. Subsequently numerous outbreaks of botulism were reported, chiefly from canned ripe olives, asparagus, spinach, corn, etc. *Bacillus botulinus* was first obtained from such sources by Thom, Edmondson, and Giltner[2] who isolated the organism from home-canned asparagus at Boise City, Idaho. Subsequently the disease and the organism producing it have been extensively investigated by Shippen,[3] Wilbur and Ophüls,[4] Dickson,[5] Burke,[6] Thom, Edmondson, and Giltner, Meyer[7] and his associates, Esty and Bengtson among others. Shippen has shown that the Nevin strain from cottage cheese is a distinct variety of *Bacillus botulinus* and Burke has divided the botulinus organisms in America into two types, Type A, the strains from canned fruits and vegetables, and Type B, the strains corresponding to the Nevin isolation from cheese. These strains produce different toxins. Finally a third type of *Bacillus botulinus* has been isolated by Bengtson[8] from fly larva, *Lucilia cæsar*. This produces a type of toxin different from both A and B. It is known as Type C.

### Bacillus botulinus Type A

These organisms have been isolated principally from canned ripe olives, spinach, corn, etc., and are the cause of the majority of cases of botulism in America. They are apparently also responsible for forage poisoning in animals, as shown by Pammel[9] and Graham,[10] and may be the cause of limberneck in poultry.

**Morphology.**—Large rods with rounded ends occurring usually in pairs or short chains, although long chains are occasionally seen (Bengtson). Kahn[11] states that they usually measure 0.3 to 0.5 $\mu$ in width and 2 to 5 $\mu$ in length. Bengtson gives the dimensions as 0.5 to 0.8 $\mu$ for the width and 3 to 8 $\mu$ for the length in twenty-four-hour cultures in meat media.

**Gram-positive** in young cultures. Old cultures soon become Gram-negative especially in meat media (Bengtson).

**Motility.**—Active from numerous long and luxuriant peritrichic flagella, varying in number from 15 to 30 or more.

**Spores.**—Usually subterminal but may be central and are slightly wider than the vegetative rods. The whole organism may swell during sporulation. The sporangia measure 0.5 to 0.7 $\mu$ in width and 2 to 9 $\mu$ in length. The spores are oval or ovoid. They have a high heat resistance. They are not destroyed by boiling water under two hours or at 95° C. under conditions which prevail in the canning industry (Dickson, Burke, and Ward[12]). Their thermal death-point is lowered in 5 per cent. acid. They are destroyed in five hours at 100° C., in forty minutes at 105° C., in six minutes at 120° C. (Weiss[13]). In acid foods they require fifty minutes at 100° C., thirty minutes at 105° C., and fifteen minutes at 110° C. for destruction (Weiss). In foods which have a lower acidity and are more nearly neutral they require ninety to one hundred and eighty minutes at 100° C., thirty to seventy minutes at 105° C., and ten to twenty minutes at 110° C. The spores of Type A are widely distributed in America. They are present in the soil of many localities as shown by Meyer. They have been obtained by Burke from moldy cherries, bush bean plants, hog manure, and moldy hay.

**Cultural Characters.**—*Glucose agar colonies* discoidal, compact, with definite outline and small, opaque knot or nucleus at periphery (Burke and Bengtson).

*Liver agar colonies* the same or diffuse. Gas production abundant.

*Plain Agar Stabs.*—Abundant growth along the line of inoculation without prolongations into the medium.

*Glucose Agar Stabs.*—Abundant line growth without prolongations into the medium. A considerable amount of gas produced.

*Liver Agar Stab.*—Abundant line growth.

*Liver agar slants* with anaërobiosis produced by pyrogallic acid. Heavy, moist growth.

*Fermentation tubes* with plain broth. Abundant growth in bulb and closed arm with a small bubble of gas. With 1 per cent. *dextrose* broth the growth is luxuriant with the production of 20 to 100 per cent. of gas in about seven days. With *liver* broth the growth is very heavy with 40 to 90 per cent. gas produced.

*Cooked Meat Medium* pH 8.5 to 7.—Marked proteolysis with darkening of the meat, gas production, and digestion (Kahn, Hall,[14] Bengtson).

*Bromcresol-purple Milk.*—Acid and gas production, precipitation of the casein and subsequent digestion (Kahn and Bengtson).

*Loeffler's Serum Stabs.*—Gas production and complete liquefaction (Kahn, Hall, Bengtson).

*Brain Medium.*—Darkening, gas production, and digestion (Kahn, Hall, Bengtson).

*Gelatin.*—Abundant growth at 37° C., gas production, and liquefaction, the gelatin remaining fluid when placed in the ice-box.

*Coagulated Egg-white Medium* pH 7.6.—Coagulation and complete digestion. Usually no gas production (Bengtson).

*Egg Fluid Medium* pH 7.6.—Coagulation and complete digestion. Usually no gas production (Bengtson).

**Proteolytic Reactions.**—Most workers, including Kahn, Hall,[14] and Bengtson, now agree that *Botulinus* Type A is highly proteolytic as indicated by the above reactions. It should be noted, however, that Reddish[15] and Kendall, Day, and Walker[16] believe that the proteolysis is due to some contaminating organisms like *Bacillus sporogenes* and that *Bacillus botulinus* is primarily saccharolytic and not proteolytic. Thus Kendall, Day, and Walker have obtained toxic strains which exhibit no proteolytic action except a softening of gelatin.

**Saccharolytic Reactions.**—There has been considerable disagreement among American observers as to the fermentative reactions of *Botulinus* Type A. Thus Thom, Edmondson, and Giltner report that the Boise City strain (Type A) when grown in a yeast water medium containing 1 per cent. of the sugar, produces slight acidity but no gas in xylose, galactose, mannite, maltose, inulin, trehalose, rhamnose, and glycerin. Considerable acidity was produced from glucose, lactose, and saccharose, gas from glucose only. Dulcite was not fermented. Armstrong, Story, and Scott[17] state that the Canton strain (Type A) ferments glucose, saccharose, lactose, and mannite with acid and gas production. Kendall, Day, and Walker, who worked with non-proteolytic strains, found that glucose, maltose, and glycerol were fermented with a slow evolution of gas. Starch was attacked by two strains and saccharose by two strains. Lactose was not affected. Hall, who had proteolytic strains, reports the fermentation of glucose, glycerol, and salicin, no action on lactose, saccharose, and inulin. Kahn who employed single-cell isolations and who had proteolytic strains of Type A found a fermentation of the monosaccharids, glucose, levulose, and galactose with acid and gas production (melizatose and glycerin also according to his table).

Bengtson who has investigated a large number of strains of Type A from single-cell isolations reports acid and gas production from dextrose, levulose, maltose, dextrin, glycerin, and salicin, dextrose and maltose showing the highest acidity. The action on dextrin was not very pronounced.

Type A toxin has in general the same properties as van Ermengem's toxin, but differs in certain particulars from Type B toxin. It is toxic to chickens in large doses like Type B. Unfiltered it is fatal to dogs in small quantities, 0.1 c.c. given subcutaneously (Graham and Erickson[18]). It is highly poisonous to man, one individual dying from tasting a small tablespoonful of spoiled corn, and another from a pod of small string beans. It may be produced and has been obtained by Dickson on media containing string beans and peas without animal protein. Starin and Dack[19] claim that the detoxified spores of Type A and Type B are capable of multiplication in the bodies of animals and produce toxin in sufficient amounts to cause experimental botulism.

Destroyed in ten minutes at 75° C. (Thom, Edmondson, and Giltner). Not destroyed by drying, retaining its strength for long periods (six months). Orr[20] states that it is destroyed at 80° C. in one-half to five minutes, at 72° C. in two to eighteen minutes, at 65° C. in ten to thirty-five minutes. Antitoxins against Type A have been prepared by Dickson and Howitt,[21] Thom and his associates, and by the Hygienic Laboratory. Their use has proved disappointing.

In men the symptoms of botulism in the cases observed in America are like those in the European cases. Ophüls and Semerak[22] believe that the lesions in botulinus are chiefly multiple thromboses of the small arteries and veins of the nervous system. These are followed by ischemic necrosis and later by inflammation. There is no direct effect on the nerve cells, the retrogressive changes being secondary and due to disturbed blood supply. Schübel,[23] Edmunds and Long,[24] and Edmunds and Keiper[25] attribute the toxicity of botulism toxin to a more or less complete paralysis of the motor nerve end-plates in the striated muscles and in the diaphragm with some impairment of other nerve endings as of the vagus. The symptoms in botulism would then be explained by the peripheral motor paralysis. Cowdry and Nicholson[26] find no microscopic evidence of any action of the toxin on the central nervous system and conclude that the peripheral nerve terminals may be affected. Coleman[27] was unable to demonstrate a combination of the toxin with brain tissue.

### Bacillus botulinus Type B

Botulinus Type B was originally isolated by Nevin from cottage cheese and was first shown by Shippen to be a distinct variety in this group. It has been isolated from a number of cases of botulism in man from home-canned goods and from oats (Graham). Disease in man from Type B is not so frequent as from Type A. Type B closely resembles Type A in morphology, motility, and cultural reactions. Kahn and Bengtson regard it as highly proteolytic, digesting cooked meat, casein, Loeffler's serum, gelatin, coagulated egg-white and egg fluid with the production of a gas with a foul odor. Bengtson states that Type B is usually somewhat more active in its proteolysis than Type A. Thus gas is produced in egg fluid medium. Nevin reports that her strain produced gas in dextrose, levulose, galactose, lactose, saccharose, maltose, mannite, dextrin, inulin, and glycerin. Bengtson has found acid and gas production from dextrose, levulose, maltose, dextrin, and glycerin, these reactions being similar to the reactions with Type A. The most decided reactions occurred with dextrose and maltose, but levulose was actively fermented by the Nevin strain. Salicin was fermented by the Nevin strain, but not by Graham's strain from oats. The spores of Type B have in general the same heat resistance as those of Type A. They are usually destroyed at 100° and 105° C. in a shorter time. Meyer finds the spores of Type B widely distributed in America, and frequently found where the spores of Type A cannot be obtained. *Botulinus* Type B grows and produces toxin at 37° C. and in sugar-free media of an acidity of +3. It can be cultivated aërobically on agar in symbiosis with a yeast. Grown with *Bacillus coli* and with a yeast in milk or with *Micrococcus aureus* in extract broth at 38° to 39° C., it kills rabbits on ingestion. It is more toxic for rabbits than the van Ermengem strain and less toxic for cats. When given on food it kills colts and donkeys by ingestion of small quantities, 0.2 c.c. of a broth culture. It is toxic for chickens only in large doses. Orr states that the toxin is more resistant than the toxin of Type A. Type B toxin is not neutralized by Type A antitoxin.

### Bacillus botulinus Type C

A third type of *Bacillus botulinus* was isolated by Bengtson from fly larva, *Lucilia cæsar*, and is known usually as the Bengtson strain. This organism produces a toxin which differs from that of Type A or Type B. It has also been obtained by Graham from the crop of a chicken with limberneck, from the liver of a chicken suffering from weakness of the legs, and from the stomach contents of a horse which died with symptoms resembling those of botulism. Morphologically the organisms resemble Type A and Type B. Motility is active, but the flagella are shorter and finer than those of A and B and less numerous, varying from 5 to 15. Spores are produced early and are terminal rather than subterminal. They are usually destroyed at 93° to 95° C. in one-half to one hour, but may resist boiling fifteen minutes. Culturally Type C differs markedly from Type A and B. While it liquefies gelatin when growth is obtained, it causes no proteolysis in cooked meat, milk, Loeffler's

serum, coagulated egg-white, and egg fluid medium. It produces acid but no gas from glucose, levulose, galactose, maltose, glycerin, and inosite. The toxin of Type C differs somewhat from A and B in its action on animals. Thus it is much more poisonous to chickens than either Type A or Type B. It is not destroyed at 75° C. in fifteen minutes, but is destroyed at 80° C. in half an hour.

### CLOSTRIDIUM PARABOTULINUM (Seddon)

Organisms belonging to the botulinus group have also been isolated from diseases of animals in South Africa and in Australia. The most important of these is the strain isolated by Seddon[28] from the bone-marrow of a fatal case of a disease in Tasmania known as "midland cattle disease." According to Pfenninger this disease is apparently identical with impaction paralysis found in Victoria, with "dry bible" in South Australia and possibly with lamziekte in South Africa. The organisms found by Seddon were called "*Bacillus parabotulinus*" by him. Pfenninger[29] and Wagner[30] have found that *Bacillus parabotulinus* of Seddon cannot be distinguished morphologically or culturally from *Bacillus botulinus* Type C. It produces a toxin which can be neutralized by Type C antitoxin, but Seddon's antitoxin from the "Seddon type" neutralizes only the Seddon toxin. *Bacillus parabotulinus* of Seddon is not agglutinated by Type C agglutinating antiserums, but Type C strains are agglutinated in low dilutions by antiserums prepared from the Seddon strain. It should be noted that there is some difference in the use of the term "parabotulinus" since Bengtson has proposed that the original van Ermengem strain be regarded as the type species and designated as *Clostridium botulinum*, Type C being grouped with this. The term *Clostridium parabotulinum* is proposed by Bengtson for the proteolytic types A and B.


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### CLOSTRIDIUM SPOROGENES (Metchnikoff)

Isolated from the intestinal contents and described by Metchnikoff[1] as Type A and Type B. Type A was found in normal dejecta, Type B in chronic colitis. One of the principle organisms found in war wounds and second in frequency in France (Henry[2]). It has probably been described under a great variety of names. It is frequently present in cultures of other anaërobes and may give rise to some of the proteolytic reactions ascribed to them. Most authors believe that cultures of the malignant edema bacillus of Koch contained this organism as the predominant form. Weinberg and Séguin[3] say that the strain of malignant edema in the Bacteriological Institute at Zurich is probably *Bacillus sporogenes*. Isolated from gas gangrene by Henry, Albrecht,[4] Muriel Robertson,[5] Barger and Dale,[6] Dean

Fig. 162.—*Bacillus sporogenes*, vegetative rods from twenty-four-hour culture in dextrose agar, stained with gentian-violet.

and Mouat,[7] Choukévitch,[8] and many others. The "Reading bacillus" of Donaldson and Joyce[9] is probably *Bacillus sporogenes* and possibly Klein's *Bacillus cadaveris sporogenes*. McIntosh and Fildes No. XI[10] is about the same as *Bacillus sporogenes*. Metchnikoff's Type A and Type B are now regarded as practically identical although the "sporogenes" in wounds corresponds more closely to Type A.

Widely distributed in war wounds, in the intestinal contents of man and animals, soil, milk, etc. Especially frequent in dust and on the exterior of the body from the waist down.

**Morphology.**—Fairly large bacillus about the size and shape of *Vibrion septique* from which it cannot be distinguished morphologically. It does not tend to form filaments in infected animals however like those of *Vibrion septique* (Weinberg and Séguin). Occurs singly, in pairs, and in chains. Ends rounded. Measures 0.6 to 0.8 $\mu$ in width and 1.8 to 4.5 $\mu$ in length (Kahn[11]), 3 to 7 $\mu$ in length (M. R. C.). In brain cultures chains are less common, but some strains tend to form long chains and filaments (Hall[12]).

Fig. 163.—*Bacillus sporogenes* from seven-day culture in plain agar, stained with gentian-violet.

Fig. 164.—*Bacillus sporogenes* from meat tube three weeks old, stained by Gram.

**Motility.**—Active in serous exudates and in young cultures. Some strains only motile at 37° C. Many cilia like those on *Vibrion septique* (Weinberg and Séguin).

**Spores** oval, usually subterminal, somewhat wider than the vegetative rods, giving the organism a characteristic clostridium appearance. Spores formed rapidly both in the tissues and in media with and without fermentable sugars. Spores may also be terminal, central, and also round (Kahn). They are very large with exceptionally thick walls. They measure 1 by 1.8 $\mu$.

Vegetative rods stained easily with the usual anilin dyes. Spores may be stained with hot Ziehl's solution.

**Resistance.**—Vegetative rods of *Bacillus sporogenes* are destroyed easily like other vegetating forms. The spores survive eight days in 5 per cent. phenol and resist a temperature of 100° C. for forty-five minutes in a capillary tube. They resist boiling one hour.

**Cultivated** easily and does not demand strict anaërobiosis. Young surface colonies transparent and irregular. Later the colonies are opaque and irregular, wooly, with raised centers and tangled filaments which grow out all around the solid center and deep into the medium. Old colonies tend to grow large and solid. Isolated colonies may be several millimeters in diameter. Deep colonies wooly.

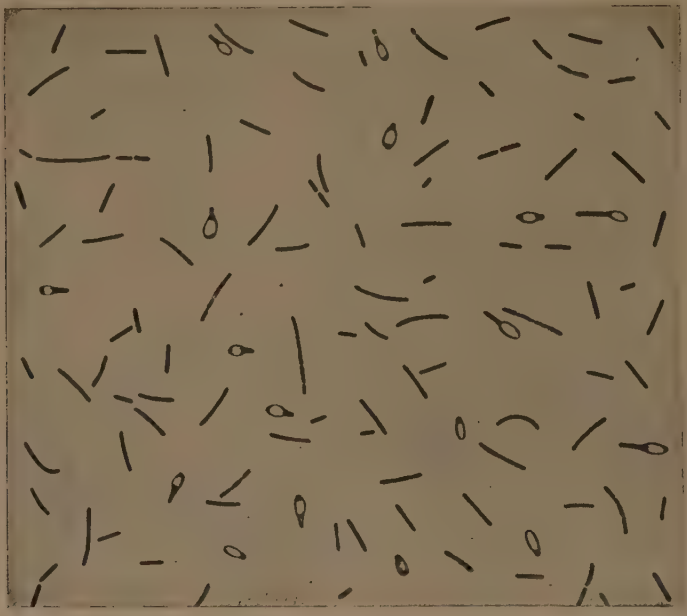

Fig. 165.—*Bacillus sporogenes* from three-week culture in dextrose agar, stained with methylene-blue.

*Deep Nitrate Glucose Agar.*—Colonies vary somewhat in size. They usually show opaque irregular centers surrounded by zones of fine filaments which may appear at one pole of the colony. There is evolution of gas and a fetid odor. Isolated colonies may be the size of a pea and vary in shape from circular to irregular ovals.

*Slant Agar.*—Colonies three to five days old are usually white but may be gray or yellow by transmitted light, opaque in center, granular from white, opaque dots. Outside lies a translucent zone spreading at the margin into fine radiating threads or roots which penetrate the medium. These threads produce a wooly appearance. Colonies hard and resistant and adhere to medium. Also moist, grayish, translucent colonies, thicker in center, margins lobulated (Adamson[13]).

*Glucose Agar.*—Colonies larger, denser, more opaque, more penetrating.

*Deep Stab.*—Gas. Wooly appearance from radiating processes. Growth 1 cm. below surface.

*Glucose Agar + Neutral Red.*—Red color destroyed. Medium orange by transmitted light, strong green fluorescence by reflected light.

*Dorset's Egg Medium.*—Moist, white surface film or isolated white colonies in twenty-

four to forty-eight hours. Later thick, moist, white layer. Medium softened and blackened. Later medium liquefied and digested. Powerful odor like strong Gorgonzola cheese.

*Blood Agar.*—Surface colonies variable according to the consistency of the medium (Hall). With stiff agar they tend to be round and dew-drop-like, with softer agar they become rhizoid, stellate, ameboid, and even confluent. Always hemolytic.

Deep 1 per cent. agar colonies are fluffy transparent balls, if well separated reaching a diameter of 1 cm. Colonies in 2 per cent. agar tend to be more restricted, opaque, and even discoid in form.

*Gelatin Stab.*—Rapid liquefaction, often complete in twenty-four hours, with flocculent colonies sinking to the bottom of the tube. Gelatin darkened (Hall). Kahn states that a trace of gas may appear, the gelatin gradually clearing; pH after 8 mo. = 7. Liquefaction due to soluble proteolytic enzyme (Kendall, Day, and Walker[14]), which appears early in cultures and causes a rapid cleavage of the gelatin.

*Inspissated Serum.*—Colonies regular, white or opaque. No radiating processes. Darkening and complete digestion in six days. Fluid clears in ten days. Foul odor. Alkaline. Final pH after 8 mo. = 8. Tyrosin and tryptophane present (Adamson).

*Cooked Codfish* (Kahn).—Gas production, blackening, and digestion. Odor foul. Crystals of tyrosin deposited. Turbidity tenacious, clearing later. pH after 8 mo. = 7.

*Brain Medium.*—Gas. Blackening. Characteristic digestion with putrid odor.

*Martin's Glucose Broth.*—Abundant turbidity and gas formation in twenty-four hours. The organisms form long filaments which sink to the bottom of the tube and the broth is slowly cleared. Odor like that of infected tissue.

*Martin's Broth without Glucose Containing Egg-white.*—Growth characters the same as in glucose broth. The egg is more or less rapidly liquefied, in twenty-four to forty-eight hours to several days. Brownish deposit at the bottom of the tube.

*Robertson's Alkaline Egg Fluid.*—Clotting gas and digestion. Odor foul. Fluid opaque. pH after 8 mo. = 7. Wolfe and Harris[15] report that there is an odor of amino-acids, with the production of ammonia and volatile acids.

*Modified Egg Water* (Kahn).—Reaction like that in Robertson's alkaline egg. Final pH = 7.2.

*Solidified Egg-albumin Cube Broth* (Kahn).—Gas. Rapid digestion. Turbidity. Foul odor. Digestion complete in sixty days. No blackening.

*Litmus Milk.*—Turned red-violet with the evolution of gas. Sometimes the proteins are digested without coagulation, sometimes a coagulation in fine particles takes place, the material settling to the bottom of the tube and being rapidly digested (Weinberg and Séguin). Wolfe and Harris find that milk shows a gas production with a foul odor. The fluid becomes turbid and has an alkaline reaction. Butyric acid, amino-acids, and ammonia are found together with small amounts of higher alcohol, butyl, or amyl. Wolfe and Telfer[16] state that the acids produced in milk consist of 17.2 per cent. butyric acid, 25.2 per cent. acetic acid, 16.1 per cent. valeric acid, and 41.4 per cent. caproic acid. No formic acid.

*Bromcresol-purple Milk* (Kahn).—Gas. Coagulation. Complete digestion in eight days, pH = 6.8 after 8 mo. Milk converted to a turbid yellow fluid.

*Muscle Broth* (Weinberg and Séguin).—Progressive digestion of the muscle fibers with the production of a black pigment.

*Cooked Meat.*—Gas, blackening, turbidity, with deposit of white, flaky crystals of tyrosin. Penetrating odor of putrefaction. Meat completely digested, turbidity persistent, pH 7.6 after 8 mo. (Kahn). Gases, carbon dioxid and hydrogen. (70 to 75 per cent. $CO_2$) (Wolfe[17]).

Volatile acids and amino-acids first formed, later ammonia, the reaction eventually becoming alkaline.

**Saccharolytic Properties.**—*Bacillus sporogenes* ferments glucose, galactose, maltose, lactose, and mannite (Bertholet[18]), glucose, levulose, maltose, galactose, and mannite (Weinberg and Séguin) with gas and acid. In 2 per cent. glucose peptone the acids consist of 41 per cent. butyric acid and 59 per cent. acetic acid (Wolfe and Telfer). Inhibition of growth occurs at a hydrogen-ion concentration of 4.94. Wolfe and Harris believe that lactose is hydrolyzed to glucose and galactose by ferment action. Henry reports fermentation of glucose, levulose, and saccharose. Kahn finds galactose, saccharose, maltose, lactose, and raffinose also. Kendall, Day, and Walker found a fermentation of glucose and maltose with acid and gas, no fermentation of lactose, saccharose, starch, and mannitol; acid with glycerol. Hall reports a fermentation of monosaccharids, not of higher carbohydrates.

**Proteolytic Properties.**—*Bacillus sporogenes* is actively proteolytic. It digests rapidly coagulated albumin, egg-white, minced meat, and brain (Hall). Media blackened from $H_2S$ and a precipitation of iron sulphid. Salmon flesh not blackened but digested. $H_2S$ is produced as shown by the addition of lead acetate. If an iron nail or certain iron salts are added the medium becomes blackened. Iron also intensifies the blackening of other media. Gas is produced from peptone water. Almost as much gas and volatile acid are produced in media without carbohydrates as with. Ammonia also produced. Polypeptids and higher nitrogenous substances are attacked, amino-acids formed and decomposed to ammonia. Butyric acid produced in quantity, together with other volatile and non-volatile acids. Reaction

remains close to neutral because of the ammonia. Odor from protein decomposition foul. No indol. Ammonia produced from erepton, and gas, amino-acids, and ammonia from alkaline casein (2 to 4 per cent.). The proteolytic action of *Bacillus sporogenes* is almost equal to that of pepsin or trypsin.

**Pathogenic Properties.**—Weinberg and Séguin claim that *Bacillus sporogenes* is pathogenic for guinea-pigs. Subcutaneous inoculation of 3 to 5 c.c. of glucose broth cultures twenty-four to forty-eight hours old produces a local lesion about the size of a pigeon's egg containing a serous putrid fluid infiltrated with gas bubbles and an extensive spreading edema. A scar forms and the lesion slowly heals. Intramuscular inoculation causes the formation of a gaseous, putrid phlegmon which may heal spontaneously or lead to the death of the animal. The hair of the skin is lost more completely than in the lesions produced by *Bacillus Welchii*. This may be explained by the local production of hydrogen sulphid.

The lesions do not differ from those produced by Muriel Robertson with her cultures identified as *Bacillus œdematis maligni*.

While Weinberg and Séguin regard *Bacillus sporogenes* as pathogenic other authors as Hilda Heller do not accept this contention. It may be noted that the organism produces a considerable amount of acid in culture media containing carbohydrates and unless this be neutralized the injection of whole cultures would produce extensive local necrosis in which degenerated tissue the organism might develop.

**Toxins.**—Weinberg and Séguin have found that the filtrate from glucose cultures twenty-four to forty-eight hours old in 3 c.c. doses is fatal to guinea-pigs in thirty to sixty seconds by intravenous inoculation. The subcutaneous injection in large doses (5 c.c.) kills guinea-pigs in a few days. In small doses an intense hemorrhagic edema is produced followed by a slough which slowly heals. Barger and Dale, working with strains identified by them as *Bacillus œdematis maligni* but regarded by Weinberg and Séguin as *Bacillus sporogenes*, found a toxin in broth cultures containing beef heart. This toxin resisted short boiling, but was destroyed by prolonged boiling, the toxicity depending upon toxic ammonium salts.

**Hemotoxins.**—Rarely strains of *Bacillus sporogenes* are feebly hemolytic to the blood of man, sheep, and guinea-pigs.

**Agglutinins.**—By treatment of rabbits it is possible to obtain a serum which agglutinates the organism in a dilution of 1 : 500.

1. Metchnikoff: Ann. de l'Inst. Pasteur, 1908, xxii, 419.
2. Henry: J. Path. and Bacteriol., 1916–17, xxi, 344.
3. Weinberg and Séguin: La gangrène gazeuse. 1917, p. 109.
4. Albrecht: Arch. f. klin. Chir., 1902, lxvii, 514.
5. Robertson: Lancet, 1916, ii, 516.
6. Barger and Dale: Brit. M. J., 1915, ii, 808.
7. Dean and Mouat: Brit. Med. Jour., 1916, i, 77.
8. Choukévitch: Ann. de l'Inst. Pasteur, 1911, xxv, 247; 345.
9. Donaldson: J. Path. and Bacteriol., 1918–19, xxii, 129.
10. McIntosh and Fildes: Rep. Brit. Med. Research Com. No. 12, 1917.
11. Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.
12. Hall: J. Infect. Dis., 1922, xxx, 445.
13. Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.
14. Kendall, Day, and Walker: J. Infect. Dis., 1922, xxx, 40.
15. Wolfe and Harris: J. Path. and Bacteriol., 1916–17, xxi, 386.
16. Wolfe and Telfer: Biochem. J. 1917, ii, 197.
17. Wolfe: J. Path. and Bacteriol., 1918–19, xxii, 270.

### Clostridium parasporogenes (McIntosh and Fildes)

Described by McIntosh and Fildes (1917) as Type XII. Practically identical with *Bacillus sporogenes* except in colony formation and specific agglutinins. Agar shake colonies are lenticular or slightly irregular in shape, not wooly as in *Bacillus sporogenes*. On inoculation into animals specific agglutinins are produced which differ from those produced by *Bacillus sporogenes*.

McIntosh and Fildes: Rep. Brit. Med. Research Com., No. 12, 1917.

### Clostridium VI (von Hibler)

Originally obtained by von Hibler from a rabbit inoculated with earth and described as No. VI.

**Morphology.**—Bacilli 0.3 to 0.6 $\mu$ wide by 1.2 to 2.4 $\mu$ in length, isolated or in pairs. More slender than *Bacillus Welchii*.

**Motility** feeble. Cilia stained by von Hibler.

**Gram-positive.**

**Spores** formed in infected muscles, but not in peritoneal exudate. Spores formed in cultures when feebly alkaline and poor in carbohydrates. Present in brain broth but not in milk. Spores very large, wider than the vegetative rods, ellipsoidal. Destroyed by heating eight minutes to 97° to 98° C.

**Cultural Characters.**—*Glucose Agar.*—Surface colonies round with irregular, tortuous contour. Deep colonies lenticular, usually regular.

*Gelatin.*—Round colonies with tortuous edges. No liquefaction.

*Milk.*—No change.

*Coagulated Serum.*—Acid reaction. No liquefaction.

*Serum* with glucose or other carbohydrates. Gas formation.

*Brain Broth.*—Medium acidified but not blackened. Abundant gas production. Odor of whey.

**Pathogenic Action.**—Virulent to guinea-pigs, white and gray rats. Subcutaneous inoculation produces hemorrhagic edema filled with gas bubbles.

von Hibler: Untersuchungen über die pathogenen Anaëroben. Jena, 1908.

### CLOSTRIDIUM VII (von Hibler)

Isolated by von Hibler[1] from the spleen of a rabbit inoculated with earth.

**Morphology.**—Bacilli about the same size as No. VI, occurring as single elements, pairs, but not in filaments.

**Motility** positive. Cilia stained by von Hibler.

**Spores** formed rarely in infected animals but found in muscle tissue after death. Spores large, ellipsoid, almost central. In cultures they are rarely present, but may be found in alkaline sheep serum and in brain broth.

**Gram-positive.**

**Cultural Characters.**—Deep agar colonies irregular with arborescent prolongations. Surface agar colonies flat, irregular, tortuous.

*Gelatin.*—Arborescent colonies. No liquefaction.

*Milk.*—No change.

*Coagulated Serum.*—Acid and abundant gas formation if glucose or other carbohydrates are added.

*Brain Broth.*—Reaction acid. Gas formation. No blackening. Odor of whey.

**Pathogenic Action.**—Virulent to guinea-pig, rabbit, cat, and pigeon.

Weinberg and Séguin[2] report that *Bacillus* No. VI and *Bacillus* No. VII of von Hibler are almost if not quite identical with *Bacillus fallax* with which the Bacillus of Stolz is also identical.

1. von Hibler: Untersuchungen über die pathogenen Anaëroben. Jena, 1908.
2. Weinberg and Séguin: La gangrène gazeuse.

### CLOSTRIDIUM X (von Hibler)

According to von Hibler[1] this organism is the type of malignant edema bacillus of Koch, Baumgarten, and Jensen. The strain described as *Bacillus œdematis maligni*[2] by Wicklein is probably the same.

**Morphology.**—Organisms measuring 0.5 to 1 $\mu$ in width by 2.4 $\mu$ in length. Long filaments both in cultures and in serous cavities of animals.

**Motility.**—Active both in serous exudates and in cultures. Cilia stained by von Hibler.

**Spores.**—Abundant sporulation in infected muscles and in all media to which it gives an alkaline reaction. It forms spores in milk and in brain broth. Spores elliptical, terminal or subterminal, rarely central.

**Staining Reactions.**—Gram-positive but organisms will not retain the stain under prolonged exposure to alcohol.

**Cultural Characters.**—*Agar.*—Surface colonies flat with irregular, tortuous edges. Deep colonies irregularly arborescent.

*Gelatin.*—Colonies with radial striations. Gelatin liquefied.

*Milk.*—Preliminary acidity followed by an alkaline reaction. No gas.

*Coagulated Serum.*—Alkaline reaction. No gas. Serum liquefied.

*Brain Broth.*—Reaction alkaline. Blackening of media. Much gas, odor putrid. Abundant spore formation.

**Pathogenic Action.**—Virulent for guinea-pig, rabbit, white mouse, white rat, gray rat, and pigeon. Small doses, $\frac{1}{5}$ to $\frac{1}{2}$ c.c., kill animals in twenty-four to forty-eight hours. Subcutaneous inoculation produces an abundant hemorrhagic edema filled with gas bubbles. Occasionally putrid. According to Weinberg and Séguin this is probably *Bacillus sporogenes*.

1. von Hibler: Untersuchungen über die pathogenen Anaëroben. Jena, 1908.
2. Wicklein: Virchow's Arch., 1891, cxxv, 75.

### CLOSTRIDIUM FALLAX (Weinberg and Séguin)

Described originally by Weinberg and Séguin[1] as *Bacillus* A. Also found by Henry[2] in cases of gas gangrene in association with other anaërobes.

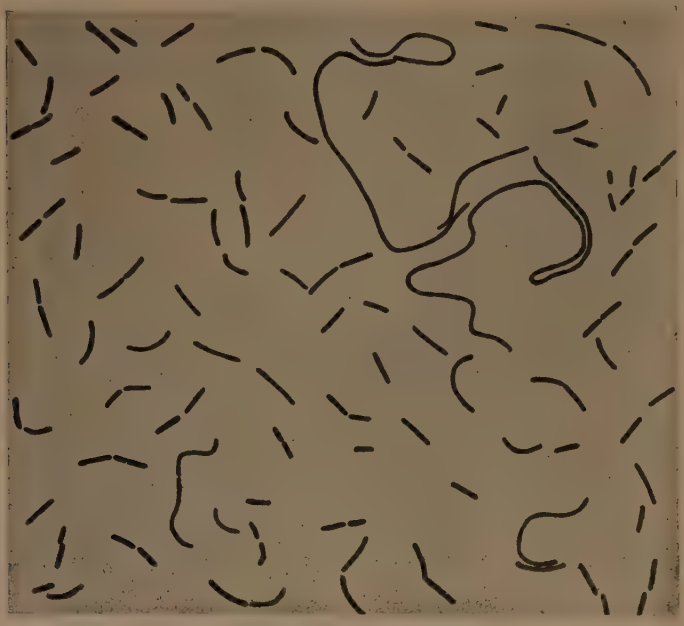

Fig. 166.—*Bacillus fallax*, vegetative rods from fourteen-day culture in dextrose agar, stained with methylene-blue.

Closely resembles the anaërobe described by Stolz,[3] but differs in the fermentation reactions. The following description taken largely from Weinberg and Séguin.[4]

**Morphology.**—Small straight bacilli with rounded ends, measuring 0.6 by 1.2 to 5 $\mu$, about the size of *Vibrion septique*. They occur singly, in pairs, and are very rarely curved. No chains.

Kahn's measurements, width 0.2 to 0.3 $\mu$, length 1.5 to 3 $\mu$, average length 2 $\mu$.

**Motility** sluggish in young, freshly isolated cultures. Actively motile in serous exudates, like *Vibrion septique*. Motility lost after several transfers. Cilia number 10 to 20 or more. They are very long, straight or spirally curved, frequently in networks.

**Spores** seldom observed by Weinberg and Séguin. Old cultures show no special resistance to boiling. Henry reports small oval subterminal spores on inspissated serum and in meat. Weinberg and Séguin have isolated one strain which formed spores poorly on Besredka and Jupille's egg medium. The majority were subterminal, oval, and the same width as the vegetative rods. Kahn[5] describes the spores as round, 1 $\mu$ in diameter.

**Capsules** in young cultures and in the peritoneal exudate of inoculated mice (Weinberg and Séguin).

**Stained** by the usual anilin dyes. Gram-positive in young cultures. In old cultures many of the forms decolorize.

**Cultural Characters.**—Surface colonies two to three days old very transparent, 1 to 2 mm. in size, with more irregular margins than colonies of *Bacillus Welchii* (Henry). Edges crenated. Larger colonies may develop refractile bubbles. Gas formed. Colonies show no tendency to form roots.

Deep colonies irregular, lenticular, or bean-shaped (M. R. C.[6]).

*Deep Glucose Nitrate Agar.*—Colonies in twenty-four hours regular, oval, or heart-shaped. Under low power of the microscope they are transparent, finely granular, amber-colored. In forty-eight hours the colonies show lateral budding, consisting of fine unequal leafy filaments. Abundant gas production, the agar being broken up as in cultures of *Bacillus Welchii*.

*Surface Growth on Agar.*—In Pinoy tubes the isolated colonies are round, slightly elevated, grayish in the center, bluish on the periphery.

*Gelatin Stab.*—Abundant growth without liquefaction (M. R. C., Kahn).

*Glucose Gelatin.*—Arborescent colonies like cotton wool. No liquefaction.

*Inspissated Serum.*—Surface colonies discrete. No liquefaction (Henry).

*Inspissated Serum Stab.*—Gas, separation of clot, no digestion (Kahn).

*Coagulated Serum* (Weinberg and Séguin).—Thin iridescent pellicle.

*Cooked Codfish Medium* (Kahn).—Small amount of gas. No other reaction.

*Cooked Meat.*—Gas production. Meat first red, then bright pink. No blackening. Fluid clears. No digestion, pH 6.5 after 8 mo. (Henry reports slight blackening and rarely a fetid odor.)

*Martin's 0.2 Per Cent. Glucose Broth.*—Abundant development, especially after repeated transfers in this medium. Turbidity dense with large quantities of gas. Deposit at the bottom of the tube due to auto-agglutination. This is ½ to 1 cm. in height, gradually sinks to the bottom and the broth clears. The broth becomes acid and gives off a non-putrid acid, sour odor a little like that of *Bacillus Welchii*.

*Martin's Broth without Sugar Containing Coagulated White of Egg.*—Abundant development, but less than in Martin's glucose broth. No digestion of the white of egg.

*Tissue Broth.*—Little development. Medium acidified.

*Egg Broth* (Besredka and Jupille).—Medium becomes turbid and acid. Egg precipitated to bottom of tube. Supernatant fluid yellow and clear.

*Modified Egg-albumin Cube Broth* (Kahn).—Slight clouding. Clearing. Few bubbles of gas. No proteolysis. Final pH 6.9 after 8 mo.

*Robertson's Alkaline Egg.*—Slow opacity (Henry). No growth (Kahn).

*Modified Egg Water* (Kahn).—Gas. No other reaction.

*Litmus Milk.*—Decolorization in twenty-four to forty-eight hours, massive coagulation after three to eight days in thermostat. The clot sinks to the bottom of the tube, its upper part riddled with gas bubbles. No digestion.

*Bromcresol-purple Milk* (Kahn).—Slow clotting, loosely organized. Few gas bubbles. Final pH 6.6 after 8 mo.

**Saccharolytic Properties.**—Henry reports that *Bacillus fallax* ferments glucose, levulose, galactose, saccharose, maltose, raffinose, mannose, xylose, starch, inulin, dextrin, glycogen, and salicin. It has no action on lactose, mannite, glycerin, and amygdalin. Lactose may be fermented (Weinberg and Séguin, Kahn). In glucose media the gas is hydrogen and carbon dioxid.

**Proteolytic Properties.**—Negative for gelatin, casein, egg-albumin, coagulated serum, and meat.

**Pathogenic Properties.**—Some strains pathogenic for guinea-pigs and mice, but not for rats. Other strains show no virulence. Following intramuscular injections in guinea-pigs, the muscles are hyperemic and infiltrated with gas and there is a wide-spread gelatinous edema also infiltrated with gas. The infection spreads into the peritoneal cavity provoking lesions like those due to *Vibrion septique*. Following subcutaneous injection an abundant gaseous phlegmon develops. Lesions in mice like those in guinea-pigs.

*Toxin.*—Filtered broth cultures kill guinea-pigs, 300 to 500 grams in weight, intravenously in doses of 1 to 2 c.c. Subcutaneous injection of 3 to 5 c.c. reproduces the characteristic edema.

*Hemotoxin.*—No action on blood-corpuscles of man, sheep, guinea-pig.

*Agglutinins.*—The serum of rabbits immunized against *Bacillus fallax* agglutinated the organism in a dilution of 1 : 500. The serum of a man dying of a general infection with the organism also agglutinated it (Weinberg and Séguin).

Because of rapid loss of pathogenicity, bacteriolytic and antitoxic sera could not be made from animals.

1. Weinberg and Séguin: Compt. rend. Soc. de biol., 1915, lxxviii, 686.
2. Henry: J. Path. and Bacteriol., 1917, xxi, 544.
3. Stolz: Beitr. z. klin. Chir., 1902, xxxiii, 72.
4. Weinberg and Séguin: La gangrène gazeuse. 1916, p. 151.
5. Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.
6. Rep. Brit. Med. Research Com. No. 39.

### CLOSTRIDIUM AËROFETIDUM (Weinberg and Séguin)

Isolated originally by Weinberg[1] and described as *Bacillus* D. Later investigated thoroughly by Weinberg and Séguin[2] and named *Bacillus aërofetidus* because of the fetid odor it produces.

Fig. 167.—*Bacillus aërofetidus*, vegetative rods from glucose agar three days old, stained by Gram.

**Morphology.**—About the same size and shape as *Bacillus fallax* in twenty-four-hour glucose broth, and in serous exudates. Henry[3] states that it is narrower than "fallax" and varies from 2 to 6 $\mu$ in length.

Non-motile or feebly motile in young cultures, actively motile in serous exudates. Kahn's dimensions are, width 0.5 to 0.7 $\mu$, length 0.8 to 2.5 $\mu$, average 1.8 $\mu$.

Spores not readily formed, subterminal when present (Bullock[4]). Kahn reports that they measure 0.4 by 0.8 $\mu$. Gram-positive in young cultures, Gram-negative in old cultures.

**Cultivated** easily on ordinary media as a strict anaërobe.

Surface colonies on serum round, transparent, 1 to 2 mm. in diameter in twenty-four hours. Deep colonies small and irregular (M. R. C.).

Henry describes the surface colonies in three days as 3 mm. in diameter, thick, semi-opaque, waxy, and round. Later they develop lateral offshoots and often a unilateral fringe.

*Deep Glucose Nitrate Agar.*—Oval colonies heart-shaped, indented, amber-colored, transparent, with lateral extensions breaking out of the indentation. Agar violently split and broken by gas production.

*Gelatin* rapidly liquefied. Large quantity of ammonia produced, amounting to a considerable proportion of the total nitrogen of the medium with a reduction in the amount of aminonitrogen (Kendall, Day, and Walker[5]); pH after 8 mo. = 6.6 (Kahn).

Soluble proteolytic enzyme secreted which produces a liquefaction without the organism. Glucose gelatin also rapidly liquefied.

*Coagulated Serum.*—Rapid and complete liquefaction with a very putrid odor.

*Martin's 0.2 Per Cent. Glucose Broth.*—Abundant turbidity in twenty-four hours. Later the broth clears and a powdery sediment forms. Odor at first slightly fetid. Broth later very turbid.

*Martin's Broth without Glucose Containing Coagulated White of Egg.*—Turbidity and powdery deposit. The white of egg is dissolved and becomes transparent after several weeks. In old cultures the egg takes on the consistence of gelatin.

*Solidified Egg-albumin Cube Broth.*—Very feeble proteolysis. Gas. Broth turbid, then clears, pH after 8 mo. = 6.6 (Kahn).

*Muscle Broth.*—Little growth, odor acid and characteristic. Tissue softened, incompletely digested.

*Cooked Meat.*—Reddened, then blackened. Putrid odor. No diminution in volume. No tyrosin crystals. Gas production (Kahn), pH = 7 after 8 mo.

*Alkaline Egg.*—Opacity and coagulation. Later a separation of the clot and a partial digestion.

*Modified Egg Water.*—Trace of gas. Soft, mushy, loosely organized clot. Feeble digestion, pH after 8 mo. = 7 (Kahn).

*Litmus Milk.*—Rapid decolorization and coagulation to a spongy clot in twenty-four hours. Casein slowly digested.

*Bromcresol-purple Milk.*—Hard acid clot, later shrunken (Kahn), pH after 8 mo. = 6.2.

*Cooked Codfish Medium.*—Slight darkening. Few bubbles of gas, pH after 8 mo. = 6.9.

**Saccharolytic properties.**—Ferments glucose, levulose, maltose, lactose, mannose, xylose, salicin, and glycogen (Henry). No action on saccharose, mannite, glycerin, inulin, and amygdalin.

**Proteolytic properties** marked. Gelatin, casein, coagulated blood-serum liquefied, egg albumin converted into a gelatinous material.

According to Kendall, Day, and Walker, in protein media containing no utilizable carbohydrate a reduction in aminonitrogen occurs in the first few days of growth. In media containing utilizable carbohydrates (glucose, lactose, or milk) the aminonitrogen increases, the amount of ammonia is less. The proteins are spared at the expense of the carbohydrates.

**Pathogenic Properties.**—Slightly virulent and only so for guinea-pigs. Five c.c. of glucose broth cultures suffice to kill guinea-pigs in two and a half to three days by intramuscular injection. At autopsy there is an extensive local gas phlegmon, somewhat putrid. Neighboring muscles are hyperemic, not gangrenous, but infiltrated with gas bubbles. Edema spreading from the site of inoculation, gelatinous and quivering, but free from gas bubbles.

*Toxins and Hemotoxins.*—Not demonstrated. The serum of rabbits immunized against *Bacillus aërofetidus agglutinates* it in a dilution of 1 : 100.

1. Weinberg: Compt. rend. Soc. de biol., 1916, lxxix, 116.
2. Weinberg and Séguin: La gangrène gazeuse, 1916, p. 161.
3. Henry: J. Path. and Bacteriol., 1916, xxi, 344. Brit. M. J., 1917, i, 806.
4. Bullock: Rep. Brit. Med. Research Com. No. 39.
5. Kendall, Day, and Walker: J. Infect. Dis., 1922, xxx, 40.

### CLOSTRIDIUM HISTOLYTICUM (Weinberg and Séguin)

First isolated as *Bacillus histolyticus* by Weinberg and Séguin,[1] and subsequently described more completely by them in gas gangrene.[2] Found also in gas gangrene by Legros and Vaucher, and by Henry.[3]

**Morphology.**—In serous exudates and in twenty-four-hour glucose broth cultures the organisms appear as diplobacilli with round ends and a clear space between the elements. Isolated individuals and chains of more than three are uncommon. Usually straight rods, rarely curved. They vary in thickness from 0.5 to 0.8 $\mu$ and in length from 3 to 5 $\mu$. In old cultures many organisms degenerate, forming fusiform and skittle-shaped involution forms (M. R. C.). Kahn[4] reports that the organisms measure 0.8 to 2 $\mu$ in length, average 1.7 $\mu$, and 0.3 $\mu$ in width. Longer and thicker rods appear in fluid media, measuring 2.5 to 6.7 $\mu$ in length. Great variations are seen here from a thin bacillus with large subterminal spores to a short oval organism with spores in the center, markedly swollen at point of sporulation.

**Motility.**—Undulatory movements like those of *Vibrion septique* in young freshly isolated cultures and in serous exudates. Motility usually lost in old cultures. Motility due to many cilia, sometimes more than twenty, often in closely wound spirals. Sporangia also motile and ciliated.

**Spores** formed readily on all media, often in two to three days as in tissue broth. They are large, oval, greater than the organisms in width, and form in the centers of the bacilli or in a subterminal position. They measure 0.5 by 1.2 $\mu$ (Kahn) and are always wider and often longer than the bacillus. Smaller spores also occur, measuring 0.3 by 0.6 $\mu$.

**Stained** by the usual anilin dyes. Gram-positive in young cultures. In old cultures the multiform degenerate forms tend to be Gram-negative. Spores stained easily by hot Ziehl's fuchsin and by chronic acid without the use of mordants.

**Cultural Characters.**—*Bacillus histolyticus* is a strict anaërobe and is somewhat difficult to isolate. It is best obtained in freshly made or freshly boiled nutrient bouillon.

*Agar.*—Surface colonies three days old 2 to 2.5 mm. in diameter, very transparent, uniform consistency, irregularly crenated margins with occasionally small offshoots (Henry). Deep colonies arborescent, coral-like, with fine wooly ends to branches. Dew-drop surface colonies on blood agar slants (Hall[5]).

*Deep Nitrate Glucose Agar.*—Young colonies, twelve to fourteen hours old, grayish yellow, about 1 to 2 mm. in diameter. Under low power of the microscope they show dense central masses surrounded by a network of fine filaments. They are somewhat like the colonies of *Bacillus sporogenes*. As the colonies age the centers become less opaque and the

Fig. 168.—*Bacillus histolyticus*, vegetative rods from glucose agar, stained by carbolfuchsin.

filamentous borders increase in size. Such arborescent colonies resemble colonies of *Bacillus edematiens*. No gas formed in agar.

*Agar Slant.*—Small, rounded, transparent colonies like those of streptococci, but grayish and less transparent in the center.

*Gelatin.*—Arborescent flocculent colonies in gelatin with and without sugar. Rapid and complete liquefaction. Kahn has found that gelatin is liquefied in twenty-four hours with a dense, white, cloudy growth. The fluid clears in twelve days. No gas. Precipitate snow-white, residue dirty slate-gray. Final pH after 8 mo. = 7.4. Kendall, Day, and Walker[6] report gas in gelatin with their strains, with a large accumulation of amino-acid, and some ammonia.

*Coagulated serum* always digested, sometimes rapidly, sometimes slowly.

*Inspissated Serum Stab.*—Blackening and digestion with a foul odor. Fluid turbid. Late feeble gas production (Kahn). Fluid clears slowly after three months. Few balls of tyrosin deposited. Final pH 7.9 after 8 mo.

*Muscle Media* (Weinberg and Séguin).—*Bacillus histolyticus* develops abundantly in physiological salt solution plus beef muscle, more abundantly in Martin's broth plus beef muscle. The muscle is rapidly attacked after three to four days in the thermostat, the

muscle-fibers divided, the connective tissue digested, the dissociated fibers forming a reddish mass in the bottom of the tube.

*Cooked Codfish Medium* (Kahn).—Blackening. Digestion two-thirds. No gas. Balls of tyrosin deposited. Digestion up to ammonia stage. Final pH = 8.4.

*Brain Medium* (Hall).—Slight darkening, slow digestion. Deposition of white crystals of tyrosin.

*Cooked Meat* (Wolfe and Harris[7]).—Gas formation. White needle-shaped crystals of tyrosin deposited. Amino-acid increased. Ammonia not increased. Some hydrogen sulphid. Large amount of volatile acid produced, 2 parts butyric acid, 1 part acetic acid. Gas consists of hydrogen and carbon dioxid, the latter usually in excess. Acid and gas from the glucosamin group in the protein molecule. According to Kahn cooked meat shows a progressive proteolysis without gas formation. Color chocolate-brown, later black. Flaky needle-like crystals of tyrosin deposited. Fluid very cloudy, then clears. Digestion complete in 8 mo. Mildly putrefactive odor. Reaction alkaline, possibly from ammonia. Final pH after 8 mo. 8.4.

*Martin's 0.2 Per Cent. Glucose Broth* (Weinberg and Séguin).—Uniform turbidity with a powdery deposit at the bottom of the tube. The tubes clear slowly, but certain strains are auto-agglutinated in twenty-four hours. Odor characteristic, moderately fetid. No gas.

*Martin's Broth without Sugar*.—Abundant development like that in sugar broth.

*Martin's Broth without Sugar Containing Coagulated White of Egg*.—Abundant and rapid development, the white of egg being slowly and completely digested.

*Solidified Egg-albumin Cube Broth* (Kahn).—No gas. Turbidity. Fragments of egg cube chipped off, some go into solution, some deposited unaltered. Fluid clear after thirty days. Digestion one-half after forty days. Final pH 7.7 after 8 mo.

*Alkaline Egg Medium* (Wolfe and Harris).—Slight proteolysis with production of amino-acids. No odor, no change in appearance. Some gas, $\frac{CO_2}{H_2} = \frac{27}{75}$. Soft diffuse coagulation (Henry). According to Kahn, Robertson's alkaline egg medium shows a soft fragile clot in eight days without gas. Digestion one-half after fourteen days with a crystal clear fluid. Remaining clot a network of fine filaments. Final pH 7 after 8 mo.

*Peptone Water* (Wolfe and Harris).—Small amount of gas, amino-acids, and a little ammonia produced. Gas = 70 per cent. $CO_2$.

*Litmus milk* decolorized in two to four days with a fine coagulum which settles to the bottom of the tube and is completely digested. In six to eight days it is converted into a clear amber-colored fluid with a barely perceptible odor. Wolfe and Harris find that acid and gas are produced in milk by *Bacillus histolyticus*, with an increase in amino-acids and ammonia. Proteolysis without offensive odor, a little like that with *Bacillus sporogenes*. Lactose slightly hydrolyzed. Gas consists of hydrogen and carbon dioxid, $\frac{CO_2}{H_2} = \frac{78.5}{21.5}$.

*Bromcresol-purple Milk* (Kahn).—Soft, mushy clot on third day. No gas. Digestion complete in ten days to clear straw-colored fluid. Final pH after 8 mo. = 7.

**Saccharolytic Properties.**—*Bacillus hystolyticus* produces acid, no gas from glucose, levulose, and maltose (Henry). Lactose slightly hydrolyzed (Wolfe and Harris[7]). No acid or gas in saccharose, salicin, glycerol. Acid with xylose and inulin (Kahn).

**Proteolytic Properties.**—*Bacillus histolyticus* liquefies gelatin, egg-albumin, casein, and coagulated serum. Henry believes that the protein substances are converted into amino-acids, the decomposition stopping at this point. Wolfe and Harris report some ammonia production in milk, and Kendall, Day, and Walker find ammonia in gelatin. Gas production from proteins slow and indolent. These last authors state that the amino-acid accumulation is large and the bacilli are not able to utilize amino-acids as they are produced.

**Pathogenic Properties.**—*Bacillus histolyticus* is virulent for the usual laboratory animals—guinea-pig, rabbit, and mouse, but less so for the rat. The virulence of different strains varies, some isolations being quite devoid of pathogenicity. Intravenous injection of 1 to 2 c.c. of twenty-four-hour cultures in Martin's broth is fatal to guinea-pigs in a few minutes, producing dyspnea and coma. Intraperitoneal injection is also fatal, the animals dying in ten to twelve hours with a hemorrhagic exudate. Intramuscular injection is followed by the production of an abundant serous hemorrhagic edema in a few hours. This goes on to a dissociation and liquefaction of the muscle-fibers, a true histolysis, as a result of which the skin and subcutaneous tissues slough off spontaneously in wide areas, involving the tibia, femur, and adjacent perineum in twelve to sixteen hours. The phlegmon is not putrid and there is no gaseous infiltration. Subcutaneous inoculation produces similar lesions, but less extensive. The lesions in rabbits and mice are like those in guinea-pigs. In rats the muscles are not attacked, the subcutaneous tissue being digested and hemorrhagic. Beckwith and Mackillop[8] have observed that intramuscular injection in guinea-pigs is followed by profound degenerative changes in liver, spleen, kidney, lung, suprarenal and heart muscle, as well as the local lesions. Chiari[9] believes that the ability to dissolve tissues is due to the toxin of *Bacillus histolyticus*.

*Toxin.*—In young cultures kept eighteen to twenty-four hours at 37° C. a toxin is present which is obtained with difficulty by filtering through a Chamberland filter, most of the toxic substance being retained in the pores. If the cultures are centrifugalized, 1 to 2 c.c. of the supernatant fluid is fatal to rabbits of 2 to 3 kilogram weight in ten minutes by intravenous inoculation. With filtered material it requires 10 c.c. to produce the same result. The filtrate has the same effect on animals as the whole culture. In the test-tube it digests white of egg slowly and liquefies gelatin rapidly. In horses injection of large amounts, 30 to 100 c.c., produces an intense local exudate.

*Hemotoxins.*—Not found for the blood-corpuscles of man, sheep, or guinea-pig.

*Antitoxins.*—The serum of horses immunized with fluid cultures freed from organisms by centrifugation neutralizes completely the toxin of the organism. The serum of horses immunized with the whole cultures only partially neutralizes these cultures. It contains an *agglutinin* for the organism active in a dilution of 1 : 400.

1. Weinberg and Séguin: Compt. rend. Acad. d. sc., 1916, clxiii, 449.
2. Weinberg and Séguin: La gangrène gazeuse, 1916, p. 165.
3. Henry: J. Path. and Bacteriol., 1916–17, xxi, 344.
4. Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.
5. Hall: J. Infect. Dis., 1922, xxx, 445.
6. Kendall, Day, and Walker: J. Infect. Dis., 1922, xxx, 40.
7. Wolfe and Harris: J. Path. and Bacteriol., 1918–19, xxii, 1.
8. Beckwith and Mackillop: J. Med. Research, 1924, xliv (n. s. xxxix), 311.
9. Chiari: Centralbl. f. Bakteriol. [etc.], 1925, xciv, 81.

### Clostridium œdematiens (Weinberg and Séguin)

Described by Weinberg and Séguin[1] as *Bacillus œdematiens*. Possibly identical with *Bacillus Novyi* (Novy's *Bacillus œdematis maligni* No. II), v. Hibler's No. V,[2] *Bacillus neigeux* of Costa and Troisier,[3] with Aschoff's gas edema bacillus,[4] and with *Bacillus bellonensis* of Sacquépée.[5] Also described by Conradi and Bieling.[6] Common in gas gangrene.

**Morphology.**—Bacilli about as broad as *Bacillus Welchii*, but somewhat longer, measuring 0.8 to 1 $\mu$ in width by 4.5 to 8 to 10 $\mu$ in length. Occasional short forms measuring 2.5 $\mu$. Ends rounded. The organisms often grow in chains of ten to twelve elements and frequently show curved or S-shaped forms. Long filaments occur rarely in young cultures, but degenerated filaments are common in old cultures. In the tissues of infected animals and man the organisms appear frequently as pairs (diplobacilli) or as short chains of four to five elements. Kahn's measurements, 1 by 3 $\mu$.[7]

**Motility.**—Progressive and undulatory in preparations free of oxygen. In the presence of oxygen the organisms are non-motile (like *Vibrion septique*). Cilia easily demonstrated by Loeffler's method. They are often very numerous, 20 or more to one organism, isolated or in bunches. Sometimes the bacilli show single terminal cilia or a twisted terminal bunch.

**Spores** are formed early in all types of cultures even those containing glucose. They appear in twenty-four to forty-eight hours and are very numerous in cultures six to eight days old. They are large, oval, measure 0.8 to 1.2 $\mu$ in breadth by 1.5 to 2 $\mu$ in length and occupy a subterminal position in the rod. The sporangia appear in the infected tissues of man and animals. The Medical Research Committee[8] state that the spores may also be central. Kahn describes the spores as always subterminal, oval, measuring 0.5 by 1 $\mu$. Kendall, Day, and Walker[9] say the spores are terminal and have spore caps so that they look like tennis rackets. These authors did not find spores in media containing utilizable carbohydrates.

**Stains** easily by ordinary anilin dyes. Gram-positive in young cultures but by the end of twenty-four hours many of the organisms lose Gram's stain. In old cultures the degenerated filaments are Gram-negative. According to Kahn the organisms often stain irregularly and have a mottled appearance.

**Resistance.**—The spores resist heating to 100° C. half an hour over the water-bath.

**Isolation.**—*Bacillus œdematiens* is a strict anaërobe and difficult to isolate in pure culture even from infected tissues. It is best obtained by cultivation in freshly prepared broth or in broth boiled fifteen to thirty minutes before using. It is best kept alive in tubes of Martin's broth prepared the same day (Weinberg and Séguin).

**Cultural Characters.**—*Agar surface* colonies flat, tend to become confluent as a surface film (M. R. C.). According to Henry[10] surface colonies measure 2 to 3 mm. in three to four days. They are opaque to granular in the center and have a semi-transparent border with a fringe of delicate offshoots. The adult colony is more symmetrical than the colony of *Bacillus aërofetidus*, is not waxy, has an opaque center, and a transparent periphery. The

marginal arborescence is fine and delicate. Weinberg and Séguin describe the agar surface colonies in Penoy tubes as very fine, flat, grayish-blue, transparent, and irregular.

*Agar Shake Cultures* (M. R. C.).—Growth delicate. Colonies snow-flake or like "bursting grenades."

*Deep Glucose Nitrate Agar.*—Deep colonies when young show opaque centers, yellowish, irregular, surrounded by irregular, short, unequal filaments, easily seen under low magnification. Later, in forty-eight hours, the colonies are clear, the centers become cloudy and obscure and the peripheries consist of abundant filaments. This clearing is coincident with the degeneration of the organisms.

*Gelatin Stab.*—Liquefaction. Kahn reports that gas is produced and the liquid gelatin clears. Amino-acids and ammonia increased (Kendall, Day, and Walker).

*Glucose Gelatin.*—Little growth in 2 per cent. glucose gelatin at 22° C. The colonies are cloudy and consist of fine arborescent filaments. The gelatin is not liquefied even after the

Fig. 169.—*Bacillus œdematiens*, vegetative rods from three-day culture in dextrose agar, stained by Gram.

lapse of four months. With 2 per cent. glucose, gelatin growth is more abundant and liquefaction is rapid.

*Litmus milk* turned red in twenty-four hours with an abundant evolution of gas. Coagulation occurs in a variable time, five days to a month, the clot being slowly broken up into fine particles. Eventually the casein is completely dissolved and a clear rose-colored fluid is produced. Kendall, Day, and Walker believe that there is no real digestion in milk. The original acid is followed by a loss in acidity due to ammonia from the intracellular use of the protein in milk. Also some increase in the amino-nitrogen ammonia. Amino-acids increased.

*Bromcresol-purple Milk* (Kahn).—Gas production. Acidity and coagulation. Fluid clears. Slight shrinking of clot. Final pH = 5.6.

*Cooked Meat Medium.*—Much gas. Meat reddened at first, then bleached, becoming lighter red, or salmon-pink. The pieces of meat become smaller but there is no reduction in total volume. Not so much gas as with *Bacillus Welchii*, more than with *Vibrion septique*. Final pH 6.6 after 8 mo.

*Coagulated Serum.*—Feeble growth, colonies flat and iridescent. No liquefaction.

*Inspissated Serum* (Kahn).—Gas. Separation of clot. No digestion. Final pH 6.8 after 8 mo.

*Cooked Codfish Medium* (Kahn).—Gas production. No other change.

*Plain Broth.*—Slight cloudiness followed by clearing.

*Martin's 0.2 Per Cent. Glucose Broth.*—Moderate turbidity in a few hours with a fila-

mentous flocculent deposit in the bottom of the tube. This is about 1 cm. in height. The cultures usually become clear in forty-eight hours, this being due to the rapid degeneration of the organisms and the abundant spore-formation. The cultures produce gas abundantly and the broth become acid to litmus. They give off a peculiar characteristic fetid odor especially when old.

*Martin's Plain Broth.*—Very little growth in Martin's broth without sugar.

*Martin's Broth without Sugar but with Coagulated White of Egg.*—Growth somewhat less abundant than in Martin's glucose broth, the white of egg not being attacked even after about a month's time.

*Tissue Broth.*—Moderate growth, the tissue being slightly softened and turning whitish. Abundant sporulation.

*Solidified Egg-albumin Cube Broth.*—Gas. No proteolysis. Fluid lears, pH 6.6 after 8 mo. (Kahn).

*Robertson's Alkaline Egg.*—Coagulation to soft, friable clot. No digestion (Henry[10]).

*Modified Egg Water* (Kahn).—Soft, mushy clot, later firm and shrunken. Gas produced.

**Saccharolytic Properties.**—Henry reports that *Bacillus œdematiens* ferments glucose, levulose, maltose, xylose, and starch. Kahn reports a fermentation of galactose, saccharose, lactose, raffinose, and mannose also, with acid in inulin. Kendall, Day, and Walker found no fermentation of starch. In glucose and glycerin these authors found an increase in acid, a decrease in amino-nitrogen and ammonia, proteins being spared at the expense of the carbohydrates.

**Proteolytic properties** slight. Gelatin liquefied, no digestion of coagulated serum, egg albumin or meat, slow action on casein. Ammonia slightly increased in all media, amino-acids in gelatin and milk.

**Pathogenic Action.**—*Bacillus œdematiens* is usually very virulent for laboratory animals, guinea-pigs, rabbits, rats, and mice. Also for sheep and horses. Guinea-pigs die in six to eighteen hours from intravenous inoculation of $\frac{1}{4}$ to 1 c.c. of twenty-four-hour glucose broth cultures from toxic strains. At autopsy the intestines and adrenals are injected and necroses are present in liver, kidney, and spleen. With subcutaneous inoculations an abundant whitish or whitish-red gelatinous edema is found. This is not infiltrated with gas and resembles the lesions in anthrax. The neighboring muscles show hyperemia and are infiltrated with numerous gas bubbles. They are not necrotic and do not give off a putrid odor. With virulent strains, as distinguished by Weinberg and Séguin from toxic strains, the lesions from subcutaneous inoculation are more extensive, the edema being gelatinous, rose-colored, and infiltrated with gas bubbles. The pathogenic action of *Bacillus œdematiens* is quite unlike that of *Vibrion septique*, the thick gelatinous edema of the former being distinguished from the thin serous exudate of the latter.

*Toxins.*—The filtrates from glucose broth cultures one to six days old are toxic to guinea-pigs in doses of $\frac{1}{40}$ to $\frac{1}{400}$ c.c. given intravenously, the strength of the toxin varying with different strains. The symptoms and lesions are much like those produced by whole cultures.

*Hemotoxins.*—Cultures are moderately hemolytic to the blood of man, sheep, and guinea-pigs.

*Antibodies.*—Immunized animals like rabbits show agglutinins against *Bacillus œdematiens* of a strength of about 1 : 100 and an antitoxin which neutralizes the toxin and whole cultures containing toxin and bacilli.

1. Weinberg and Séguin: Compt. rend. Soc. de biol., 1915, lxxviii, 274; 507.
———: La gangrène gazeuse. 1916, p. 132.
2. v. Hibler: Untersuchungen über die pathogenen Anaëroben. Jena, 1908.
3. Costa and Troisier: Compt. rend. Soc. de biol., 1915, xxxviii, 552.
4. Aschoff, Fraenkel, Konigsfeld, and Frankenthal: Deutsche med. Wchnschr., 1916, xvi, 469.
5. Sacquépée: Compt. rend. Soc. de biol., 1915, lxxviii, 316.
6. Conradi and Bieling: München. med. Wchnschr., 1916, lxxiii, 113.
7. Kahn: J. Med. Research, 1922, xliii (n. s. xxxviii), 155.
8. Rep. Brit. Med. Research Com. No. 39.
9. Kendall, Day, and Walker: J. Infect. Dis., 1922, xxx, 141.
10. Henry: J. Path. and Bacteriol. [etc.], 1916–17, xxi, 344.

### CLOSTRIDIUM MULTIFERMENTANS Stoddard

Described originally by Stoddard[1] as *Bacillus multifermentans tenalbus* from a case of gas gangrene. Fairly strict anaërobe.

**Morphology.**—More slender than *Bacillus Welchii*, of variable length, short forms tending to be curved, long forms showing sinuous distortions. Ends very rounded, occasionally almost conical. Resembles *Vibrion septique* in morphology but under similar conditions of

culture has a larger proportion of short and swollen forms. Gram-positive in young cultures. Quickly becomes Gram-negative, usually Gram-negative in two to three days. A twenty-four-hour culture shows all variations from Gram-positive to Gram-negative organisms. Usually granular and feebly refractive. No capsules. Occasionally end-to-end. Long chains not found. Long threads produced on agar. Early sporulating forms tend to stick together in dense masses. Swollen forms and spores rapidly produced on egg medium, but also occur in broth, milk, and on agar. In spore formation the bacillus swells centrally with a gradual tapering off to the ends. Gram-negative at this stage and faintly staining, especially at end where the spore is produced. A deeply staining Gram-positive mass appears at the opposite end. Spore large, oval, refractile, subterminal, the rest of the bacillus gradually disintegrating. A twenty-four-hour culture in milk shows practically only swollen and sporulating bacilli. Bacillus may also become pear-shaped, barrel-shaped, or may elongate. Spherical chromatic dots often appear at both ends. Decolorization irregular, Gram-positive dots and bands remaining. Like *Vibrion septique*. Swollen and irregular forms resemble the "citrons" usually believed to be characteristic of *Vibrion septique*. Subterminal and central spores may develop quickly in bacilli before they have swollen and become Gram-negative.

**Motility** very active in condensation water of egg slant, less marked in chopped meat. Organisms actively motile in plain bouillon, non-motile in glucose bouillon.

**Cultural Characters.**—*Glucose agar* surface colonies in twenty-four hours grayish, 2 to 3 mm. in diameter, raised, with sharp edges and somewhat irregular outline. A hand lens shows a clear translucent stratum in which white specks float. They form triangular or other straight-sided geometrical figures and thus present a crystalline appearance. Older colonies (three days) are dense, opaque, clear white with narrow translucent rims which may still show crystalline flecks, often arranged radially. Colonies are sticky, stringing out when touched but not adherent. Old colonies may be 5 mm. in diameter, thick, heaped-up, sides rather steep, edges sharp, and clear. Color opaque, clear white.

*Deep glucose agar* colonies small, white, opaque, irregular, often lenticular with a horn-like projection from one side, or reniform with a projection from the "hilus." They are 1½ mm. in size. Older colonies, forty-eight hours, large with large gas bubbles which tear up the agar giving a confluent growth about their periphery. Often the growth extends as a white film on the under surface of the agar.

*Blood Agar*.—Surface colonies at first smaller and more opaque than on glucose agar, and do not show clearly the crystalline flecks. They tend to be flatter, not to increase in size and to become level with the agar. Definite hemolytic zone. Deep colonies have the same shape as deep glucose agar colonies but become Vandyke brown in color, thus standing out sharply from the pale yellowish-red agar.

*Colonies on egg medium* (whole egg and tryptic broth inspissated in slants) in twenty-four hours are 1 to 2½ mm., raised, yellow, with sharp irregular edges. They are very coherent and elastic, adhering closely to the medium. On the second or third day the colonies are larger, gray or white, with a narrow, colorless, or light yellow border. They are now sticky, but not so adherent or elastic. Colonies increase in diameter up to 4 to 6 mm. 2 to 3 mm. thick, pure opaque, white in color. No blackening or digestion of the egg.

*Chopped Meat*.—Gas in twenty-four hours. No blackening or digestion. No change in color. No liquefaction of gelatin or coagulated serum.

*Glucose Bouillon*.—Heavy flocculent precipitate in twenty-four hours, cloudiness from small, floating specks. Precipitate easily broken up on shaking, then quickly settles. Gas appears throughout the culture when shaken.

*Plain Broth*.—Diffusely cloudy with a slight somewhat flocculent sediment.

*Milk*.—Consistency thickened in twenty-four hours apparently from precipitation of casein with the evolution of gas which produces a coarsely bubbly foam, ¼ to ½ inch deep, over the surface. Later the casein precipitates more completely and settles to the bottom leaving a cloudy fluid above. With a heavy inoculation the reaction may resemble that due to *Bacillus Welchii*, but the clot is softer and easily broken up.

The British Medical Research Committee[2] reports that *Bacillus multifermentans tenalbus* ferments glucose, saccharose, lactose, maltose, raffinose, glycerin, inulin, and starch, but not mannite or dulcite.

Non-pathogenic to rabbits and guinea-pigs.

1. Stoddard: Lancet, 1919, i, 12.
2. Rep. Brit. Med. Research Com. No. 39.

### ADAMSON'S CENTRAL-SPORED ANAËROBE

Isolated from putrefying meat and from the intestines of a hippopotamus by Adamson[1]. Apparently identical with McIntosh and Fildes Type XII.[2] Somewhat like *Bacillus sporogenes*.

**Morphology.**—(From agar.) Actively motile. Gram-positive bacillus larger and thicker than *Bacillus sporogenes*. Rods often curved. Ends round. Single or short chains or filaments. Oval spores larger than the spores of *Bacillus sporogenes*, little or no wider than bacillus, central or subterminal. Long, curved, twisted filaments on glucose agar. Also clubbed involution forms. On Dorset's egg medium the bacilli are more slender, spores central and abundant.

**Cultural Characters.**—*Agar colonies* small, circular, moist, translucent, later opaque and whitish. Margins indented. Many colonies may have short, thin processes. Non-penetrating. No fine radiating processes.

*Alkaline Agar.*—Colonies small, translucent.

*Glucose Agar.*—Growth slow, colonies small, rather thick, moist, translucent or almost transparent, smooth margins.

*Dorset's Egg.*—Slow digestion and complete blackening. Odor fetid, less cheese-like than with *Bacillus sporogenes*.

*Egg Broth.*—Blackened and digested. Clotted, clot broken later. More intense blackening at bottom than with *Bacillus sporogenes*.

*White of Egg Broth.*—No liquefaction.

*Brain Medium.*—Slight blackening. Some digestion. Alkaline.

*Inspissated Serum.*—Colonies first transparent, thin, whitish, and moist. They may coalesce to form a surface film. Slow liquefaction. Later non-liquefied portion darkened. Strong, offensive odor. Crystals of tyrosin on surface.

*Broth.*—Turbidity with offensive odor.

*Glucose Broth.*—Scanty growth. Gas production.

*Gelatin.*—Rapid and complete liquefaction. Turbidity, offensive odor.

*Meat.*—Reddened first, then blackened. Gas. Little or partial digestion.

*Milk.*—No acid. Digestion. Small clot. Alkaline reaction.

*Fermentation* of glucose and maltose with acid and gas. No fermentation of saccharose, lactose, or mannite.

1. Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.
2. McIntosh and Fildes: Rep. Brit. Med. Research, Com. No. 12.

### HEMPEL'S PROTEOLYTIC ANAËROBE

Found by Hilda Hempel in two war wounds. Somewhat resembles *Bacillus sporogenes* of Metchnikoff.

**Morphology.**—Bacillus with rounded ends, three to six times as long as broad. May or may not retain Gram's stain. Oval, subterminal spores. Bacilli with spores at either end are not uncommon, especially in meat cultures. Moderately motile.

**Cultural Reactions.**—No acid or gas from any carbohydrates. Gelatin liquefied. Inspissated serum rapidly, but not completely digested. Dorset's egg medium softened, but not liquefied. Meat turned bright terra-cotta, but not blackened.

Hempel: J. Hyg., 1918, xvii, 13.

### CLOSTRIDIUM TETANI (Nicolaier)

Discovered by Nicolaier[1] in animals, mice, guinea-pigs, and rabbits inoculated with garden earth and developing tetanus. Cultivated in mixed cultures on coagulated sheep serum. Similar organisms found by Rosenbach in pus in a patient with tetanus following frost-bite. Obtained in pure culture by Kitasato[2] by anaërobic methods and shown to be the cause of the disease. Usually called *Bacillus tetani*.

**Morphology.**—In gelatin cultures a few days old fine bacilli, 2 to 4 $\mu$ long and 0.3 to 0.5 $\mu$ wide, with slightly rounded ends. The organisms are present as individual cells, short or long slightly curved threads, joined as V's or lying parallel. In cultures six to eight days old the number of rods is less, and there are more threads. In cultures ten to fourteen days old the rods and threads are gone and only spores appear.

**Motility** definite, but not very active, due to peritrichic flagella, 30 in number according to Kanthack and Connel,[3] 50 to 100 according to Votteler.[4]

**Spore-formation.**—Spores begin to form at 37° C. in twenty-four to forty-eight hours,

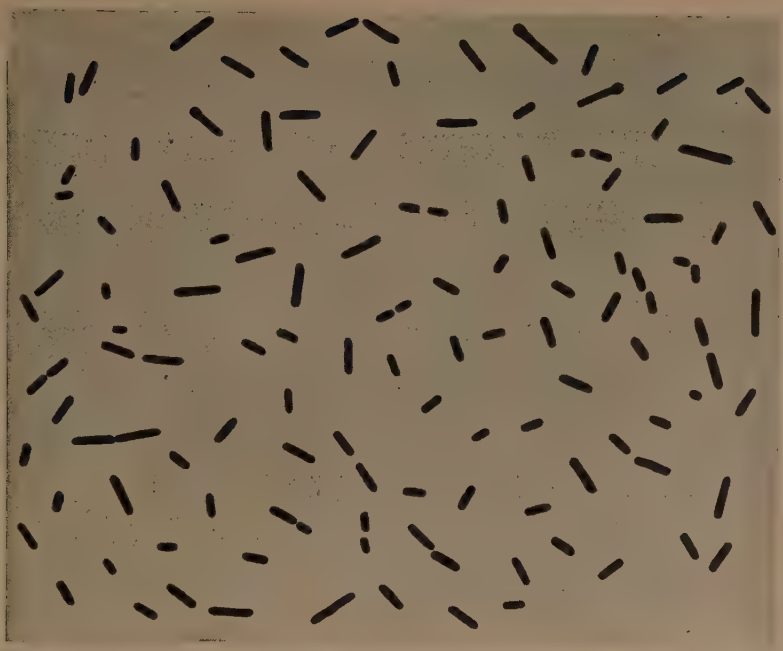

Fig. 170.—*Bacillus tetani*, vegetative forms from forty-eight-hour culture in plain agar stab, stained with gentian-violet.

Fig. 171.—*Bacillus tetani*, eleven-day culture in plain agar stab, stained with gentian-violet.

in gelatin at room temperature in eight to ten days. They are formed early in blood-serum and in sugar-free broth. The organisms which produce spores enlarge at one pole and the spores begin to develop just inside the outer wall. As the spores enlarge they move outside and later take up a position absolutely at the end of the rod. Such forms resemble drumsticks or glass-headed pins. The spores measure 1 to 1.5 $\mu$ in diameter. With sugar or glycerin in the media the spores become elliptical (v. Hibler). In old cultures only spore-bearing organisms and free spores are seen. Spores are formed in the tissues of infected animals and man.

**Staining Reactions.**—Stains with ordinary anilin dyes. Gram-positive. Spores demonstrated by Ziehl's solution followed by 25 per cent. $H_2SO_4$. Flagella stained best by van Ermengem's method, with young cultures twelve to sixteen hours old. Sporulating rods lose motility and are Gram-negative.

**Methods of Cultivation.**—Obtained first by Kitasato by growing mixed cultures on slant agar one to two days at 37° C., heating to 80° C. one hour to kill off all vegetative bacteria, transferring to fluid agar, which is then allowed to solidify in an atmosphere of hydrogen. This method is usually successful when a large number of tetanus bacilli are present and the other organisms do not produce resistant spores. Isolation can also be accomplished in broth under anaërobic conditions at 37° C., by repeated heating to kill off non-sporulating bacteria. Von Hibler[6] reports that tetanus bacilli can be separated from edema bacilli by deep cultures in coagulated rabbit's blood kept at 37° C. eight days. They can be separated from *Bacillus Welchii* by cultivation in milk eight days. After this time tetanus spores are produced, but not gas bacillus spores. By heating, a pure culture of *Bacillus tetani* can be obtained (v. Hibler[6]). The organisms can also be gotten by cultivating in deep stab cultures (Liborius[7]), and fishing suspicious colonies by sterile glass pipets. The media should be boiled before use to drive off the oxygen and cooled quickly. It is not necessary to replace the oxygen by hydrogen as the organism develops in the depths of media kept under aërobic conditions. The tetanus bacilli after isolation grow on ordinary media under anaërobic conditions. The oxygen may be absorbed by alkaline pyrogallic acid or may be driven off by hydrogen. In old cultures the growth takes place more readily, and it is only necessary to cover the inoculated medium with a layer of oil or of sterile medium. It also grows in symbiosis with aërobic bacteria which may absorb the oxygen (Pasteur), or in media to which a piece of animal tissue is added (Smith, Tarozzi). Various substances added to media favor the growth, such as 2 per cent. glucose, 0.3 to 0.5 per cent. sodium formate, 1 per cent. sodium indigosulphate (Kitasato), and litmus solution (Novy).

Optimum temperature 37° C. No growth below 14° C.

*Agar Plates.*—Colonies appear after two to three days at 37° C. as fine clouds, under low power a network of very fine threads, the so-called "medusa-head." Surface colonies usually small, 1 mm. in diameter in forty-eight to seventy-two hours.

*Agar Stab.*—Growth along the line of inoculation below the surface with radiating branches extending into the agar at right angles. The agar stab is frequently likened to an inverted fir-tree. The line growth may appear fluffy or cottony and is quite heavy in old cultures.

*Blood agar* colonies sometimes opaque and lenticular with few or no filamentous outgrowths, sometimes diffuse and hazy. Still others are like minute transparent dew-drops (Adamson,[8] Hall[9]).

*Glucose agar shake* colonies delicate filamentous outgrowths spreading from a small central nucleus, 2 mm. in diameter in forty-eight hours (M. R. C.[10]). Agar acid with bubbles of gas.

*Glucose Agar Stab.*—Growth more abundant than in plain agar and is usually visible in twenty-four hours. It presents the same system of outgrowths radiating from a central line. The agar is turned acid and bubbles of gas appear along the line of growth. Sometimes a very distinct precipitate appears in the agar.

*Gelatin Plates* (at 20° C.).—Colonies visible on the third day. Under low power they have a compact central part from which thin threads radiate. According to Sanfelice the colonies resemble colonies of *Proteus mirabilis*. In many strains the peripheral zones show a more star-shaped structure, so that they resemble colonies of the hay bacillus (Kitasato). In deep gelatin shake cultures the colonies are of both types, compact radiating and looser dendritic.

*Gelatin Stab.*—Growth begins about 2 cm. below the surface. From the line of growth numerous fine processes extend almost at right angles into the gelatin, the whole growth giving an appearance of a cloudy turbidity. The gelatin is broken up by numerous gas bubbles. Liquefaction takes place slowly as a rule and is complete after ten to fourteen days. According to Hall the gelatin is also blackened. Certain strains effect merely a lowering of the solidification point so that the gelatin hardens in the ice-chest and remains fluid at room temperature. Kendall, Day, and Walker[11] report that gelatin is not liquefied, shows a limited evolution of gas, and a gradual increase in titratable acidity.

*Cooked Meat.*—Gas, turbidity, later clearing somewhat, slate-gray in color. Feeble proteolysis. Meat darkened. Digestion one-third (Kahn[12]). Fluid turbid after 8 mo.

pH = 7.2. Some crystals deposited, not tyrosin. Odor foul, not so marked as with *Bacillus sporogenes*.

*Brain medium* blackened very slowly from $H_2S$ (Hall). This reaction is seen only in old cultures and is accelerated by the addition of an iron nail.

*Inspissated Serum* (Kahn).—Few gas bubbles. Slight darkening to a dirty-gray. Feeble proteolysis about one-quarter. Fluid clears. Slight odor of putrefaction. Final pH = 7.4 after 8 mo.

*Cooked Codfish Medium* (Kahn).—Gas. Blackening. Slight odor of putrefaction. Some digestion.

*Robertson's Alkaline Egg* (Kahn).—Soft, mushy clot and feeble proteolysis. Narrow band of clear, watery fluid develops. No gas. Later fluid opalescent above, turbid below. Faint odor of putrefaction. Final pH 6.9 after 8 mo.

*Solidified Egg-albumin Cube Broth* (Kahn).—Gas, digestion two-thirds. Fluid turbid, then clears. Final pH 7 after 8 mo. Digestion peculiar, disintegrated substance remaining as a kind of halo about the cube of egg.

*Milk*.—Growth varies. Sometimes no visible changes are apparent, sometimes after prolonged incubation a white flocculent precipitate forms or a fairly solid coagulum suggesting the action of a rennet-like ferment. Slight gas production. According to Kendall, Day, and Walker there is a gradual increase of titratable acidity with a slow and limited evolution of gas, probably from the proteins in the milk. Hall states that milk cultures may become alkaline and that the casein is not digested.

*Bromcresol-purple Milk* (Kahn).—Little gas, hard, acid clot. Slight digestion, one-quarter. Fluid clear, watery. Final pH 6.4 after 8 mo.

*Broth*.—Growth excellent with the production of a uniform diffuse turbidity in twenty-four to forty-eight hours.

*Potato*.—No growth usually. Vaillard and Vincent report a fine expansion like the characteristic growth of *Bacillus typhosus*. Microscopic examination reveals long threads. No spores.

Poor growth usually on blood-serum (Kitasato) which is not liquefied (M. R. C.). Tizzoni[13] and Kitt report a liquefaction of serum.

Good development in rabbit blood (Tizzoni, Cattani, and Baquis[14]), aërobic growth and spore production.

**Saccharolytic Properties.**—Considerable difference of opinion exists as to the ability of *Bacillus tetani* to decompose carbohydrates. Most authors state that it ferments dextrose in twenty-four to forty-eight hours with a moderate evolution of gas consisting of carbon dioxid, hydrogen sulphid, and methyl mercaptan. An acid reaction is produced and an offensive odor like that of putrefying organic material. Kahn states that acid and gas are produced in abundance from glucose, levulose, and galactose, much acid and a few bubbles of gas from saccharose, maltose, lactose, raffinose, mannose, and xylose. Years ago Achalme[15] claimed that *Bacillus tetani* does not ferment the carbohydrates. This is the opinion also of the British Medical Research Committee, Hall, and Kendall, Day, and Walker.

**Proteolytic Properties.**—Feeble. Gelatin is liquefied and slowly blackened with a production of $H_2S$. Coagulated albumin is slowly softened and slightly digested, never distinctly liquefied. Very slight softening of cooked meat and brain. Casein is not noticeably attacked. Gas appears in various protein media, chiefly carbon dioxid, due to feeble proteolysis. Kendall, Day, and Walker believe that a moderate production of ammonia and a small but definite decrease of amino-nitrogen are the only nitrogenous changes. Indol produced in media containing tryptophane (Smith[16]). Cultures give off volatile substances which may be fatty acids and develop a disagreeable odor like burnt hoofs (horn). Traces of acid may be formed in sugar-free media.

**Resistance.**—Spores survive a long time under natural conditions when protected from sunlight. Henrijean[17] was able to produce tetanus with a splinter of wood infected eleven years previously. Spores destroyed by direct sunlight with some rapidity. According to Vaillard and Vincent[18] organisms dried on paper and exposed during the summer to direct sunlight with the temperature not above 35° F. lose virulence and ability to form spores in six days. Kitasato states that tetanus spores are destroyed by streaming steam in five minutes, by 5 per cent. carbolic acid in fifteen hours, by 5 per cent. carbolic acid plus 0.5 per cent. HCl in two hours, by 1 per cent. sublimate in three hours, by 1 per cent. sublimate plus 0.5 per cent. HCl in thirty minutes. Park and Williams state that tetanus spores are killed in ten minutes by exposure to live steam at 105° C., by sublimate 1 : 1000 in three hours, by sublimate 1 : 1000 plus 0.5 per cent. HCl in thirty minutes, by silver nitrate in 1 per cent. solution in one minute, and in 1 : 1000 solution in five minutes.

**Distribution.**—Tetanus spores enjoy the widest distribution. They are present in soil in practically every part of the world where careful examinations have been made. They have been isolated from water from many different areas. Different types of soil from the same locality are not equally infected. Thus Nicolaier showed that samples of soil from woods were not so much infected as soil from gardens, streets, and plowed fields. Heavily manured pastures are more infected than virgin soils. The organism is present frequently

on the outer clothing, in street dust, in the dust of the floors of dwelling houses. Whether any regions are free from tetanus bacilli is difficult to say, but certain areas exist where reported cases of tetanus are rare. In America the number of cases is by no means as great as before the prophylactic use of tetanus antitoxin, but the disease appears to be especially common in certain states, as Louisiana. Tetanus spores have been isolated from various parts of the human body, from the dirt about the feet (Uhlenhuth and Haendel), from the hair of the head, from carious teeth (Schiff, Lurett). They have been obtained from the dejecta in 5 per cent. of examinations by Pizzini. Recently TenBroeck and Bauer[19] have reported their wide distribution in China, finding them in many instances in human dejecta. They are especially common in the dejecta of animals. Found by Park, Williams, and Krumwiede in the intestine in 15 per cent. of the horses and calves in the vicinity of New York in 1915. Sanchez, Toledo, and Veillon found them in 5 per cent. of their examinations. Joseph found them constantly in the intestines of healthy cattle. In all probability animals are largely responsible for the wide distribution of the tetanus bacillus. Present in horse and cow manure. Occasionally found in vaccine virus, commercial gelatin (Levy and Bruns, Andersen[20]), cat-gut sutures, etc.

**Pathogenic Action.**—Following inoculation with tetanus bacilli tetanus develops in a large series of susceptible animals. At autopsy the local changes are limited to hyperemia and edema and the bacilli are present in very small numbers. They are difficult to obtain from the blood and organs, but may be demonstrated by the use of large quantities of material, especially if this material itself is kept at 37° C. for a few days (Oettingen and Zumpe,[21] v. Hibler). The pathogenic action of the tetanus bacillus is due to an extracellular toxin. Spores washed free of toxin or deprived of it by heating to 75° to 80° C. are in general unable to produce tetanus unless proper conditions for their development are present, such as injury to the tissues. By protection from leukocytes, as in sacks made from filter paper (Berzelius), the organisms are able to multiply and produce their poison. Infection cannot be caused by feeding or inhalation unless the alimentary canal or the lungs are injured. If injurious substances like sulphuric or sulphurous acid are employed to produce bronchial catarrh the inhalation of toxin-free spores produces tetanus only when lesions of the tissues exist. In all instances the tetanus produced by the toxin corresponds to the tetanus produced by the living organisms.

**Tetanus Toxin.**—The tetanus toxin was first demonstrated by Behring and Kitasato in broth cultures and found to produce the disease in all susceptible animals. It is best obtained by cultivating the organism in broth under anaërobic conditions. The broth should be freshly prepared, neutral or slightly alkaline, and contain 1 per cent. peptone and 0.5 per cent. NaCl. Sugar and glycerin should not be added to the medium, since the acid produced injures the toxin, as do higher concentrations of salt (Knorr[22]). The broth becomes turbid in twenty-four hours, the turbidity increasing up to about eight days and a sediment developing. The cultures give off a disagreeable odor of fatty acid. Maximum toxin production is found on the tenth day, after which the toxicity of the broth decreases. The broth is then filtered or centrifugalized free from bacilli and spores. It can also be sterilized by the addition of 0.5 per cent. carbolic acid or toluol. The toxin can be precipitated by phosphate of lime or aluminum phosphate (Vaillard and Vincent), by zinc chlorid (Brieger and Boer[23]), or by a saturated solution of ammonium sulphate (Brieger and Fraenkel[24]). The ammonium sulphate precipitate is filtered, dried, and then dissolved in water and dialyzed against distilled water to get rid of the traces of ammonium sulphate. The final fluid is concentrated "in vacuo" or evaporated at 22° C. and dried in a desiccator (Tizzoni). The toxin is thrown down almost completely by ammonium sulphate. Thus the maximum toxicity of a broth culture may be as high as 0.000005 c.c. as a fatal dose for a mouse of 10-gram weight. By precipitation with ammonium sulphate preparations can be obtained in which 0.0000001 gram is a fatal dose for a mouse.

The tetanus toxin is very labile, easily destroyed by physical and chemical agencies. It is injured at 40° C. and by high salt concentrations of the medium, 3 per cent. It deteriorates rapidly in the ice-box when exposed to the air, but less quickly when covered by toluol or liquid paraffin. In filtrates it is destroyed in five minutes at 65° C., in twenty minutes at 60° C. (Kitasato). This destruction may not be complete (Vaillard and Rouget), complete destruction taking place only at 80° C. in three hours. Destroyed in direct sunlight in fifteen to eighteen hours (Kitasato), eight to ten hours (Fermi and Pernassi). The dried tetanus poison withstands a temperature of 120° C. one hour and exposure to light one hundred hours. The poison is destroyed by electric current of 0.5 ampere strength in two hours.

It is destroyed in one hour by 0.55 per cent. HCl, 0.735 per cent. $H_2SO_4$, 1.63 per cent. phosphoric acid, 10 per cent. acetic acid, 6 per cent. butyric acid, 4 per cent. lactic acid, 0.3 per cent. sodium hydrate, 0.42 per cent. potassium hydrate, 1 per cent. quicklime.

It is injured by trichloriodid (Behring and Knorr) in 0.01 to 0.05 per cent. solutions. The injured toxins retain their poisonous properties unchanged for months.

Von Lingelsheim regards tetanus toxin as probably a colloid which does not belong to the proteins since specific protein precipitants do not throw it down (Brieger and Cohn[25]).

The purest double zinc salts of the toxin prepared by Brieger and Boer did not give the xanthoprotein, biuret, or Adamkiewicz reactions. Hayaski[26] believes that the tetanus toxin belongs to the albumoses.

Tetanus toxin is not produced except in traces when the organisms are grown on Uschinsky's medium. It is produced in mixed cultures of *Bacillus tetani* with other bacteria, *Bacillus subtilis* or *Bacillus mesentericus*, under aërobic conditions (Debrand).

Park and Williams believe that the best method for the preparation of tetanus toxin is by the use of stock cultures of the tetanus bacillus in semisolid agar neutral to phenolphthalein, a method devised by Wilcox[27] and modified by Anderson and Leake.[28] From those stock cultures preliminary cultivations of the tetanus bacillus are made in glucose broth freshly heated in the air and then cooled down to 50° C. The tetanus bacillus is grown for several generations in this broth (two to seven). The flasks for toxin production are inoculated with about 5 c.c. of the broth from these active cultures. The flasks are incubated in the dark for fifteen days at 36° to 37° C., after which period the tetanus toxin is present in its maximum amount. From this time on it deteriorates rapidly.

**Pathogenic Action of Tetanus Toxin.**—In small animals, mice, guinea-pigs, or rabbits, subcutaneous or intramuscular injection of toxin after an incubation period of one to three days is followed by cramps in the neighboring muscles. These increase in severity and are soon followed by the same phenomenon in muscles of the opposite side, other groups of muscles in other parts of the body then becoming involved. This type of tetanus beginning locally is known as *ascending tetanus* in contradistinction to the tetanus seen in man and large domestic animals, *descending tetanus*, developing independently of the site of inoculation. The length of the period of incubation and the extent of involvement vary with the animal injected. Estimations of the strength of the tetanus toxin were originally made by comparison with the effect on mice. Thus von Behring injected varying quantities of toxin, always diluted to 0.25 c.c., into mice of uniform weight. When general tetanus of the muscles developed and death occurred in four and a half days the amount of toxin was regarded as a single fatal dose (Limes +). When practically no symptoms developed from the amount inoculated beyond a slight wrinkling of the skin the amount injected was regarded as Limes 0. The difference between Limes 0 and Limes + is the differential strength of the toxin (Differenzwert Dwert). In freshly prepared toxins this is 3 to 5, that is one-third to one-fifth of the fatal dose is just capable of producing symptoms. The tetanus poison has the same action on all the individuals of the same species. It varies greatly in its capacity to produce disease with different species. The horse is twelve times as sensitive as the mouse in proportion to its body weight. The mouse is 30,000 times as sensitive as the hen. The hedgehog (Igel) is extremely resistant. Tetanus of fowls is characterized by an increase in reflex activity and a more or less uniform involvement of the musculature. Sparrows are more sensitive than other birds (Fermi and Pernossi[29]). Frogs are slightly sensitive. This is not shown at ordinary temperatures, only when the animals are kept at 37° C. Vincent finds that an increase of sensitiveness is shown by guinea-pigs kept at high temperatures. Serpents and beetles are quite refractory. According to Metchnikoff[30] reptiles (alligators) are not poisoned by tetanus toxin, but bind it and produce antitoxin. By direct injection into the brain tetanus can be produced with a shortening of the period of incubation and the development of different symptomatology, *cerebral tetanus*. Intraperitoneal injection does not lead to characteristic tetanus as a general rule. In intravenous injection in small animals the incubation period is longer and larger doses are required to produce fatal results. The entire musculature of the body is involved. In rabbits non-fatal doses produce a disease described as tetanus without cramps (Dönitz), mild rigidity of the muscles, and fatal cachexia. Similar effects have been observed by Vaillard in guinea-pigs. Alimentary administration is without effect. This is ascribed by Ransom[31] to a failure of absorption on the part of the alimentary mucosa and not to destruction of the toxin by the epithelial cells or the digestive juices. This was not confirmed by Carrière. The digestive juices, acid stomach juice (Rabinovitsch[32]), bile (Vincent[33]), and pancreatic juice destroy tetanus toxin "in vitro" and also in the alimentary canal.

The incubation period depends upon the amount of toxin injected and the place of application. It is shorter with large doses, but never can be done away with completely. Toxins injured by trichlorid of iodin have longer incubation periods. The period is shorter the more sensitive the animal is to the toxin. In horses it is nine to twelve days, in man, five to fourteen days.

The tetanus toxin contains two different ingredients, the substance which is responsible for the lesions and symptoms of the disease, and an accessory substance which has a special action on the blood-corpuscles. These are known as tetanospasmin and tetanolysin.

**Mechanism of Action of Tetanospasmin.**—It has been shown by Morax and Marie[34] that the tetanus toxin is absorbed by the substance of the nerves and subsequently by Meyer and Ranson[35] that it is taken up by the motor end apparatus and travels centripetally by the axis-cylinder, of the motor nerves, first to the spinal ganglia of the affected side, then of the other side, and then to the brain. The toxin in the blood finds its way to the central nervous system through the motor nerves. Meyer and Ranson believe that the sensory

nerves do not take part in the conduction of the toxin because of the presence of the spinal ganglion cells which present a barrier to the poison. The direct union of the tetanus toxin with the substance of the brain has been demonstrated by Wassermann and Takaki.[36] According to Marie and Tiffenau[37] it is probably the albuminoid-like substances in the brain which combine with the toxin.

**Tetanolysin.**—In addition to tetanospasmin broth cultures of *Bacillus tetani* contain a substance acting on blood-corpuscles and dissolving them. This has been shown by Ehrlich to be a substance distinct from the tetanospasmin. It dissolves the red blood-corpuscles of rabbits, goats, sheep, horses, and other animals. Madsen[38] has proved that tetanolysin is neutralized by antitoxin just as diphtheria toxin is neutralized by diphtheria antitoxin and consists of prototoxin, deuterotoxin, tritotoxin, and toxone. The tetanolysin plays no definite rôle in natural or experimental tetanus.

**Tetanus Antitoxin.**—Artificial immunization of animals to the tetanus toxin was first accomplished by Behring and Kitasato[39] who showed that the resistance of immunized rabbits was due to substances circulating in the blood-stream. Since then the tetanus antitoxin has been studied by many observers and the methods of its preparation have been perfected. In v. Behring's first experiments horses were immunized with tetanus toxin containing trichloriodid, the amount of this substance being diminished with each injection till pure toxin was given. Roux and Martin employed toxins weakened by Lugol's solution, 1 : 500. Babes and Pawlowsky utilized toxin-antitoxin mixtures with a slight excess of toxin, increasing the amount of toxin with the succeeding doses. In addition to horses other animals can be immunized and their serum contains appreciable amounts of tetanus antitoxin. Treated animals are actively immune to doses of tetanus toxin which will kill normal untreated animals. The tissues themselves, however, are hypersensitive to the toxin and are protected from it by the circulating antitoxin. Horses produce more antitoxin in response to treatment than either dogs or rabbits. In Europe the strength of the antitoxin is estimated in antitoxin units, a unit being the quantity of antitoxin which will neutralize 40,000,000 fatal doses for mice.

In America standardization of the antitoxin is now practised on guinea-pigs according to the procedure advised by Rosenau and Anderson[40] or some modification of it. The minimum lethal dose of toxin is determined on 350-gram guinea-pigs, being the amount which will kill a pig of this weight in four days with characteristic symptoms. A "test dose" of toxin contains 100 minimum lethal doses. The unit of antitoxin is the quantity which saves the life of a guinea-pig of standard weight when given with an official test dose of toxin. In the standardization of the antitoxin the L+ dose represents the amount of toxin which kills a guinea-pig of 350-gram weight when mixed with 0.1 unit of antitoxin instead of 1 unit as in diphtheria. Suitable dilutions of the antitoxin are made, mixed with L+ doses and injected into guinea-pigs. The smallest amount of serum which neutralizes the L+ dose so that the animals do not die for four days (ninety-six hours) represents 0.1 antitoxic unit and ten times this amount represents 1 antitoxic unit.

METHOD OF TITRATING TETANUS ANTITOXIN

(Rosenau and Anderson)

| Number of pig. | Weight of pig in grams. | Subcutaneous injection of a mixture of: | | Time of death. |
|---|---|---|---|---|
| | | Toxin (test dose). | Antitoxin. | |
| | | Gram. | C.c. | |
| 1..... | 360 | 0.0006 | 0.001 | Two days, four hours. |
| 2..... | 350 | 0.0006 | 0.0015 | Four days, one hour. |
| 3..... | 350 | 0.0006 | 0.002 | Symptoms. |
| 4..... | 360 | 0.0006 | 0.0025 | Slight symptoms. |
| 5..... | 350 | 0.0006 | 0.003 | No symptoms. |

In this series the animal receiving 0.0015 c.c. of antitoxin died in approximately four days; this amount of serum, therefore, represents 1/10 of a unit.

Tetanus antitoxin is used almost exclusively for prophylactic purposes for punctured wounds, especially where there is a good deal of maceration of the tissue. The dose varies from 500 to 1500 units, although larger quantities (5000 units) are occasionally employed.

Tetanus is common in man, horses, cattle, and sheep, rare in dogs and goats. In the tropics tetanus neonatorum from infection of the umbilical cord is common (Anders and

Morgan[41]). Puerperal tetanus is rare, but was more common in early times. Tetanus usually follows injuries in which the tissues are macerated, like gunshot wounds, etc. The tetanus bacillus was frequently isolated from war wounds in France. The organisms may be isolated from the lesions, but usually with some difficulty. At autopsy they appear rarely in the spleen and heart's blood (Hochsinger,[42] Hohlbeck,[43] Creite[44]). With the prophylactic use of tetanus antitoxin the number of cases of tetanus in man and animals has become much less.


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### CLOSTRIDIUM PSEUDOTETANICUM (Sanfelici)

Organisms like *Bacillus tetani*, but less toxic, were isolated by Sanfelici from meat infusion and earth. Morphologically, bacilli of various length with terminal round spores. Colonies on agar like those of *Bacillus tetani*. Growth in agar, gelatin, milk, and in media containing starch like the growth of the tetanus bacillus. Originally described as Anaërobe IX.

Sanfelici: Ztschr. f. Hyg., 1893, xiv, 372.

### CLOSTRIDIUM PUTRIFICUM (Bienstock)

Originally described as *Bacillus putrificus coli* by Bienstock[1] who isolated it from the intestinal tract of a human cadaver. It has been investigated especially by Tissier and Martelly[2] in decompositions of meat and by Metchnikoff[3] in the intestinal contents. Considerable difference of opinion exists as to the cultural properties of this organism. The present description is based on the observations of Tissier and Martelly, Weinberg and Séguin,[4] and Reddish and Rettger,[5] who agree that it has no action on carbohydrates and decomposes proteins actively. Organisms similar in morphology and fermenting carbohydrates should be regarded as different species. *Bacillus putrificus* of Bienstock is probably identical with Klein's *Bacillus cadaveris sporogenes*, Distaso's *Bacillus putrificus filamentosus*.[6] Rodella's No. II or No. III[7] and v. Hibler's No. XIV may be the same.[8] *Bacillus putrificus* was found by Weinberg and Séguin several times in cases of putrid gas gangrene.

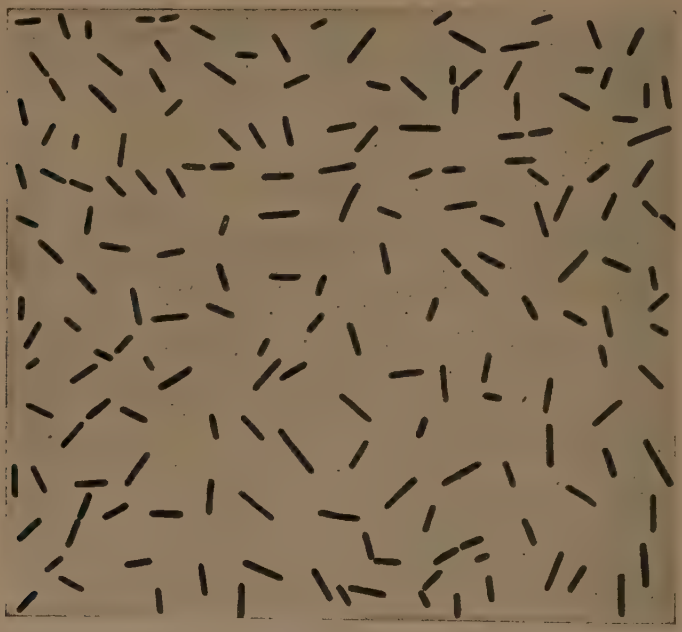

Fig. 172.—*Bacillus putrificus*, from three-day culture in dextrose agar stab, stained with gentian-violet.

**Morphology.**—Straight, thin rods in twenty-four-hour broth cultures, measuring 0.6 to 0.8 $\mu$ in width by 5 to 6 $\mu$ in length. Often quite curved, in old cultures frequently in filaments (Reddish and Rettger).

**Motility** active in young cultures especially in egg-meat (Reddish and Rettger). Motility slower but not completely lost during sporulation. Cilia stained by v. Hibler.

**Spores** formed readily particularly in egg-meat. According to v. Hibler sporulation is favored by an alkaline reaction of the medium. Spores are large in proportion to the size of the rod, are round or oval, and situated at the extreme end of the rod like the spores of *Bacillus tetani*. The rods and spores look like "drumsticks." Spores not observed by Reddish and Rettger in glucose agar or glucose broth.

**Stains** well with the usual anilin dyes. Gram-positive in young cultures. In old cultures the organisms tend to be Gram-negative.

Spores resist heating one to two minutes.

**Cultivated** only under anaërobic conditions. Growth at temperatures from 22° to 37° C.

*Glucose Agar* (1 Per Cent.).—Surface colonies at 37° C. are small, delicate, and almost transparent (Reddish and Rettger). Edges irregular, not deeply indented. No outgrowth, no apparent fringe. Somewhat, but not coarsely, granular. Colonies develop only after about three days and are best observed with a hand-lens.

*Deep Glucose Agar* (1 Per cent.).—Fine colonies appear in three to four days in the lower portions of the tube (Reddish and Rettger). They have dense nuclei and fine, but not long hair-like radiations from the central portions. Weinberg and Séguin state that deep glucose agar colonies are characteristic in forty-eight hours, irregular, embossed, resembling chestnut burrs, showing fine prolongations from single areas or from the entire surface. Later the colonies become confluent and appear to be composed of central nuclei surrounded by fine arborizations.

*Deep Plain Agar*.—Development slower than in glucose agar. The colonies lie farther from the surface due possibly to the absence of reducing substances.

*Surface Agar Colonies*.—Under anaërobic conditions, fine, bluish, transparent expansions.

*Gelatin colonies* have long hairy prolongations (Weinberg and Séguin). The medium is slowly softened and liquefied at the end of four to five days. Reddish and Rettger state that gelatin is rapidly liquefied by old cultures and slowly by young cultures, requiring three to four weeks. Liquefaction progresses at the same rate in plain and glucose gelatin.

Fig. 173.—*Bacillus putrificus* from six-day culture in broth, stained with gentian-violet.

*Loeffler's Serum*.—Growth in three days at 37° C. from heavy inoculation with old cultures. Complete digestion after five days (Reddish and Rettger). Weinberg and Séguin report that coagulated blood-serum becomes alkaline and is liquefied without gas production.

*Plain Broth*.—Turbidity in twenty-four hours with a fine granular sediment. The broth gives off a fetid odor.

*Glucose broth* shows the same turbidity and sediment.

*Brain Broth*.—Alkaline reaction with a blackening of the medium. Abundant gas formation and slightly putrid odor (Weinberg and Séguin).

*Milk*.—Under anaërobic conditions the milk becomes rose-colored or yellow-ochre-colored in four to five days (Weinberg and Séguin). It then gradually becomes transparent. The superficial cream layer shows fine granules of casein which gradually precipitate to the bottom of the tube. The serum turns clear yellow-green and the casein is completely digested. Old milk cultures have the odor of old cheese. Reddish and Rettger state that the casein is slowly precipitated and digested in one to four days with an old inoculum, in about four weeks with a young inoculum. The more rapid digestion may be due to preformed ferments carried over.

*Egg-meat* (Reddish and Rettger).—Slight softening of the solid matter in seven to ten days, the meat and egg particles becoming finer. Pronounced reddening. Digestion very

apparent by the end of the second week with the evolution of foul odors. Meat darkened very slightly and remains red until decomposition is complete when there is a definite browning. Residue in old cultures (two to three months) almost black. No gas. The evaporation of the fluid keeps pace with the slow digestion.

Weinberg and Séguin have observed turbidity and gas in media containing muscle or fibrin with or without sugar. The cultures give off a fetid odor. The solid particles are slowly dissolved leaving a powdery, blackish residue.

**Saccharolytic Properties.**—No acid or gas with any carbohydrates. Reddish and Rettger find that glucose is consumed only very slightly, less than 0.1 per cent., not enough to indicate any saccharolytic action.

**Proteolytic Properties.**—Weinberg and Séguin find that *Bacillus putrificus* secretes a lipase which emulsifies and saponifies fats in media containing muscle. It rapidly transforms native proteins. It decomposes fibrin, producing hydrogen sulphid, but no phenol or indol as first pointed out by Bienstock and Wallach, together with proteoses, amins, leucin, valerianic acid and paraoxyphenyl-propionic acid. At the end of three weeks with 30 grams of fibrin to start with there is present but 0.2 per cent. insoluble albumin, 0.1285 per cent. proteoses and 0.7 per cent. extractive. This action is due to a *tryptic diastase* which may be isolated and which will completely digest 30 grams of fibrin in three weeks. This action is inhibited by acid. The ferment itself produces no gas, no fatty or aromatic acids, no phenol, but proteoses in abundance. Amins, leucin, and tyrosin are also present. The other products mentioned above are due to the action of the bacilli on the proteoses. It also digests coagulated white of egg and coagulated blood-serum. Reddish and Rettger say that albumoses, peptone, and higher polypeptids are attacked by *Bacillus putrificus* on the third to the eighth day with a decrease in biuret-giving substances and an increase in ammonia and amino-acids. Action delayed. Amino-nitrogen almost completely converted into ammonia-nitrogen in egg-meat and protein media.

**Non-pathogenic.**—No toxin. No hemotoxin.

**Varieties.**—Several varieties of *Bacillus putrificus* have been described. One of these which coagulates milk without digesting it is called *Bacillus paraputrificus* by Bienstock. Kimura[9] has found six strains of *Bacillus putrificus* which differ from each other and from *Bacillus tetani* serologically.

1. Bienstock: Ann. d. l'Inst. Pasteur, 1906, xx, 407.
2. Tissier and Martelly: Ann. de l'Inst. Pasteur, 1902, xvi, 865.
3. Metchnikoff: Ann. de l'Inst. Pasteur, 1908, xxii, 929.
4. Weinberg and Séguin: La gangrène gazeuse. 1916, p. 119.
5. Reddish and Rettger: J. Bact., 1923, viii, 375.
———: Sturges and Rettger: J. Bact., 1919, iv, 97.
6. Distaso: Centralbl. f. Bakteriol. [etc.], 1911, lxix, 97.
7. Rodella: Ztschr. f. Hyg., 1902, xxxix, 201.
———: Ann. de l'Inst. Pasteur, 1905, xix, 804.
8. v. Hibler: Untersuchungen über die pathogenen Anaëroben., Jena, 1908.
9. Kimura: Centralbl. f. Bakteriol. [etc.], 1924, xcii, 342.

### Clostridium pseudotetanus (Tavel)

An anaërobic organism like *Bacillus tetani* found by Tavel[1] in abscesses associated with the intestinal tract. It measured 0.5 $\mu$ in breadth by 5 to 7 $\mu$ in length. Terminal spores oval and not spherical like tetanus spores. Spores destroyed at 80° C. Motile flagella, 4 to 12. No pathogenic action.

Tavel described this organism as closely similar to Bienstock's *Bacillus putrificus coli*, but an obligate anaërobe, while Bienstock's bacillus was a facultative anaërobe. Weinberg and Séguin[2] believe that this pseudotetanus bacillus is either Bienstock's *Bacillus putrificus* or Henry's *Bacillus tertius*.

1. Tavel: Centralbl. f. Bakteriol. [etc.], 1898, xxiii, 538.
2. Weinberg and Séguin: La gangrène gazeuse. p. 124.

### Clostridium tetanomorphum (McIntosh and Fildes)

Described by McIntosh and Fildes[1] as Type IX or *Bacillus pseudotetanicus*. Closely resembles *Bacillus tetani* in morphology. Named by the British Medical Research Committee. Frequently found in wounds, especially in late infections, often with the tetanus bacillus.

Tavel's *pseudotetanus* bacillus,[2] Debono's *Bacillus anaërobius alcaligenes*,[3] Fleming's bacillus,[4] and Adamson and Cutler's bacillus[5] may be identical or closely related. Resembles also Sanfelici's[6] pseudotetanus organisms.

**Morphology.**—Gram-positive, highly motile rods. Spores spherical, terminal. Formed on all media in twenty-four to thirty-six hours. Hall[7] states that the spores are oval, may also be subterminal, and are formed in media not containing monosaccharids.

**Cultivation.**—Grows best in alkaline media such as meat media (M. R. C.[8]). Grows in mixture with *Bacillus sporogenes* later than *sporogenes* which prepares the way for *tetanomorphus*.

*Surface colonies* on serum agar plates and slants are delicate and flat with slightly crenated edges. The organisms tend to grow as a continuous surface film (M. R. C.).

*Deep agar colonies* small, opaque, irregular points, not wooly or branched.

*Surface colonies* on blood agar are minute dew-drop-like points (Hall). They may also unite to form a spreading confluent growth.

*Gelatin.*—Abundant gas formation in twenty-four hours. Gelatin not liquefied according to the British Medical Research Committee. Hall reports that gelatin is softened and after four to five days' growth remains fluid in the ice-chest. No blackening of gelatin.

*Meat Medium.*—Meat particles turned pink, no blackening or digestion. No blackening even when an iron nail is added. No $H_2S$.

*Brain Medium.*—No blackening or digestion.

*Milk.*—No change.

Gas produced in all media which support growth (Hall).

*Glucose* and *maltose* fermented. No fermentation of levulose, galactose, saccharose, lactose, salicin, mannite, inulin, glycerin, or dulcite (M. R. C.).

**Non-pathogenic.**

Agglutinins for the organism may be produced in rabbits (M. R. C.). Organism not agglutinated by a strong tetanus agglutinating serum.

1. McIntosh and Fildes: Rep. Brit. Med. Research Com., No. 12, 1917, p. 32.
2. Tavel: Centralbl. f. Bakteriol. [etc.], 1898, xxiii, 538.
3. Debono: Centralbl. f. Bakteriol. [etc.], 1912, lxii, 229.
4. Fleming: Lancet, 1915, ii, 376.
5. Adamson and Cutler: Lancet, 1907, i, 688.
6. Sanfelici: Ztschr. f. Hyg., 1893, xiii, 339.
7. Hall: J. Infect. Dis., 1922, xxx, 445.
8. Rep. Brit. Med. Research Com., No. 39.

### CLOSTRIDIUM TETANOIDES A (Adamson)

Found by Adamson[1] in war wounds, discharges from war wounds, blood-cultures, garden earth, and pasture soil. Named *Bacillus tetanoides* A.

Not like Sanfelici's No. IX or like MacIntosh's organism with terminal round spores. Best cultivated on glucose agar. Clostridium spores.

**Morphology.**—From glucose agar the organisms vary in size, 4 to 12 $\mu$ in length, 1 to 2 $\mu$ thick. Average size 6 to 7 $\mu$ by 1 to 1.5 $\mu$. Isolated, pairs, or chains of three to five. No filaments. Round ends. Tapering, cigar, or spindle-shaped. Young cultures Gram-positive. Old cultures after thirty-six to forty-eight hours Gram-negative. Irregular banded staining. Young or non-sporing individuals show clear refractile unstained areas crossed by bands, especially when stained by carbol-thionin-blue or methylene-blue. Spores large, round, considerably wider than the bacillus. Subterminal with polar caps. Spores form in three to five days on glucose agar. Spores Gram-positive even when bacillus is Gram-negative. Many involution forms on Dorset's egg medium, pear-shaped, or fat spindle-shaped. Multiplication by budding from small coccus-like bodies.

**Motile** in broth cultures.

**Cultural Characters.**—*Glucose agar colonies* circular, regular, opaque, bluish- or grayish-white with moist, shining surface. Whole colony thick and raised except when old, when it has conical raised centers. Colonies sticky, tenacious, can be pulled out into threads, do not penetrate the medium. Also translucent or almost transparent very thin, film-like colonies, circular or slightly lobed or indented. On moist surface these colonies show translucent radiating streaks regularly and beautifully arranged, like frosted glass. These dendritic streaks often radiate from a center.

*Glucose Agar Slant.*—Thick, opaque surface film.

*Glucose Agar Stab.*—Thick line growth $\frac{1}{2}$ cm. below surface with short rounded outgrowths, but no marked penetration of medium. No gas.

*Glucose Neutral Red Agar.*—Color changed to orange by transmitted light, bright green fluorescence by reflected light.

*Plain Agar.*—Less active growth than on glucose agar. Moist, flat translucent colonies thicker in center, circular, or irregularly indented. Also less abundant dendritic streak-like growths.

*Agar Slant.*—Translucent film with scattered, thicker portions yellow by transmitted light. Not so sticky or elastic.

*Alkaline Agar* (−10 Eyre's Scale[2]).—Colonies rather more opaque and always smaller.

*Dorset's Egg Medium.*—Young cultures show flat yellow-white colonies, later large, white, dry, penetrating the medium to depth of 1 mm. Colonies firm and hard. Coalescent colonies form firm resistant crust. No digestion, liquefaction, or discoloration.

*Egg Broth.*—Little growth. Soft clot.

*White of Egg Broth.*—Little but no marked digestion. Some production of indol.

*Brain Medium.*—No discoloration or digestion. No change in reaction. Occasionally gas with transient pink color.

*Inspissated Serum.*—Scanty growth only on moist serum. No digestion or liquefaction. Thin, film-like surface colonies which coalesce.

*Acid Broth.*—Vigorous growth. Most abundant in +5 broth Eyre's scale. Reaction becomes alkaline in broth with trace of indol.

*Glucose Broth.*—Abundant turbidity with precipitate and clearing. Deposit thick, mucus-like, and sticky. Most abundant growth in acid glucose broth.

*Gelatin.*—No liquefaction. Narrow line growth.

*Meat.*—No change. No digestion.

*Milk.*—Very slight alkalinity. After five to seven days a precipitation of casein, translucent fluid above.

Glucose and maltose fermented with very slight acid. No gas. Some strains fail to produce acid. No acid with saccharose or mannite. No acid from starch. No digestion of cellulose. No digestion or liquefaction of fat.

1. Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.
2. Eyre: Bacteriological Technique. 2. ed., 1913.

### CLOSTRIDIUM TETANOIDES B (Adamson)

Found by Adamson in putrefying meat, but not in war wounds. Closely resembles *Bacillus putrificus*, but has less marked proteolytic activities. Described as *Bacillus tetanoides* B.

**Morphology.**—Rather large bacilli on plain agar, of medium thickness and of somewhat variable length, straight or frequently curved. Isolated organisms, pairs, chains, and curved filaments. Spores formed readily, round, terminal. Morphology like *Bacillus tetani*, but spores are smaller. Strongly Gram-positive even in old cultures. In old cultures on alkaline agar may be Gram-negative. In brain medium very long, twisted, coiled filaments, strongly Gram-positive. Short and fat in broth, slightly motile. Involution forms on inspissated serum.

**Cultural Reactions.**—*Plain agar* colonies circular, flat when young, later thickened, moist, opaque or semi-opaque, white or creamy. Margins more translucent and organisms show tendency to spread as a translucent film. No offshoots and no penetration of medium.

*Alkaline Agar.*—Growth less profuse. Colonies smaller, thinner, and remain translucent.

*Glucose Agar.*—Colonies quite opaque and thick. Growth more active than on plain agar. Less tendency to spread as superficial halo.

*Glucose Agar Stab.*—Line growth without radiation or penetration of medium. No gas.

*Dorset's Egg.*—Growth slow. Colorless surface film. After eight to ten days medium softened and eventually partially liquefied. No blackening or discoloration. No complete breaking up of medium

*Egg Broth.*—Coagulation. No discoloration. Clot may shrink slightly with a clear surrounding liquid.

*White of Egg Broth.*—Slow digestion. After ten days substance rendered translucent, subsequently slowly dissolved. No pigment.

*Brain Medium.*—No change. No gas. No blackening. No digestion. Reaction becomes slightly alkaline.

*Broth.*—Scanty turbidity and precipitate. Growth most active in acid broth.

*Glucose Broth.*—Growth more active than in plain broth, deposited at bottom in five to six days.

*No indol.*

*Inspissated Serum.*—Transparent surface film becoming gray in old cultures. Slow softening. Partial, never complete, liquefaction.

*Meat.*—No digestion. Slight reddening.

*Gelatin.*—Slowly liquefied. Complete liquefaction only after six to eight days' growth in incubator.

*Milk.*—Rendered slightly alkaline. Precipitate of casein after some days, upper layers translucent or almost clear. Digestion slight if any.

No acid from sugars or starch.

Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.

### CLOSTRIDIUM TETANOIDES E (Adamson)

Found by Adamson in 2 cases of war wounds and described as *Bacillus tetanoides* E.

**Morphology.**—On agar a very small bacillus, short and often rather thick, often tapering toward one or both ends. On glucose agar the organisms are longer, more slender, and many involution forms are produced. These are large, swollen as wide as the length of the bacillus, round, oval, or pear-shaped, and finely granular. In white of egg broth the bacilli are more elongated, isolated, in pairs or short chains. Involution forms appear like those on glucose agar. Spores produced readily, small, round, almost terminal in position, causing a swelling at one end of the organism, tad-pole in form. Spores form after thirty-six hours in alkaline agar, usually after forty-eight hours in other media.

**Motility** feeble.

**Stained** with usual anilin dyes. Gram-negative even in young cultures. On Dorset's egg medium Gram-positive spots are found in the organism. Involution forms stain yellow brown with safranin, not stained with methylene-blue or thionin blue. Sporangia may be Gram-positive.

**Cultural Characters.**—*Agar colonies* small, circular, without offshoots. Young colonies whitish, translucent. Old colonies opaque and almost waxy. Colonies white or slightly tinged with yellow.

*Glucose Agar.*—Colonies larger than on plain agar. Growth more profuse. Colonies opaque, circular, or diffuse, spreading, translucent, rather watery, with irregular outline.

*Deep Glucose Agar Stab.*—Thick, opaque growth with gas bubbles.

*Dorset's Egg Medium.*—Small, transparent, whitish colonies. No digestion.

*Gelatin* not liquefied.

*Inspissated Serum.*—Small, moist colorless colonies or moist surface film. No change in the medium. No liquefaction.

*Brain Medium.*—Small amount of gas. No other change.

*Broth.*—Moderate growth. Turbidity, clearing, and precipitate. Growth better in neutral or slightly acid broth than in strongly acid or alkaline broth.

*Glucose Broth.*—Small amount of gas and an acid reaction.

*White of Egg Broth.*—No digestion.

*Meat Medium.*—No change.

*Milk* slowly acidified. Not clotted.

Acid and gas in glucose, maltose, lactose, and mannite. No action on saccharose.

Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.

### CLOSTRIDIUM SPHENOIDES (Douglas, Fleming, and Colebrook)

Isolated originally by Douglas, Fleming and Colebrook, from war wounds. Named "sphenoides" because of the wedge-shaped morphology of the sporulating rods. Description from the British Medical Research Committee[1] and Hall,[2] who studied a strain of Douglas, Fleming, and Colebrook from the Lister Institute.

**Morphology.**—Small, motile bacillus, fusiform in non-sporing stage. Arranged in pairs, placed end-to-end like Hoffman's pseudodiphtheria bacillus, also in short chains (Hall). Gram-positive when young, rapidly becoming Gram-negative.

**Spores** first subterminal, then terminal as they mature. They are large, round or almost round (Hall), broader than the bacillus. The body of the bacillus with the spore becomes wedge-shaped with the point of the wedge at the ends opposite the spore. In older degenerated forms the organism looks like a drumstick, the end opposite the spore always remaining pointed. Spores formed abundantly, especially in media not containing fermentable carbohydrates in excess, notably in blood agar cultures under alkaline pyrogallol (Hall).

**Cultural Characters.**—Surface colonies on agar 1 mm. in diameter, round, with smooth edges or occasionally slight irregularities (M. R. C.).

*Blood agar* surface colonies at first like minute dew-drops, becoming whitish and somewhat opaque when older. Hemolytic (Hall).

*Deep 1 Per Cent. Agar.*—Isolated colonies look like minute opaque disks (Hall).

*Meat Medium.*—No change in color. Little gas (M. R. C.).

*Brain Medium.*—Turbidity and gas with little or no change in consistency of the tissue (Hall). No blackening even in presence of added iron. $H_2S$ apparently not set free.

*Gelatin.*—Gas production and blackening (Hall). No liquefaction.

*Coagulated Serum.*—No change.

*Milk.*—Acid and usually a soft clot (M. R. C.). Gas production without coagulation noted by Hall. No digestion of casein.

*Broth.*—Profuse, even turbidity. No indol (Hall).

**Saccharolytic Properties.**—According to the British Medical Research Committee three of their strains fermented with acid and gas glucose, maltose, galactose, lactose, and salicin, while two fermented also mannite, saccharose, dextrin, and starch. Hall noted the fermentation of glucose, lactose, and salicin and no fermentation of saccharose, inulin, and glycerin.

**Proteolytic properties** completely lacking.

**Non-pathogenic** to guinea-pigs and rabbits.

Found only in a few cases which had had gas gangrene.

1. Rep. Brit. Med. Research Com. No. 39.
2. Hall: J. Infect. Dis., 1922, xxx, 445.

### CLOSTRIDIUM TERTIUM (Henry)

Probably described first by Fleming as Bacillus Y[1] and probably identical with v. Hibler's No. IX[2] (originally called No. XI by v. Hibler), Rodella's No. III[3] and Robertson's end-spored anaërobe.[4] Possibly some of the

Fig. 174.—*Bacillus tertius* from nine-day culture in dextrose agar showing threads, stained with gentian-violet.

organisms called "amylobacter" are also *Bacillus tertius*. Third in frequency in war wounds in France, and therefore named *Bacillus tertius* by Henry.[5] Found in infant stools by Hall and Matsumura.

**Morphology.**—Long, thin bacilli, often slightly curved, with a beaded or granular appearance. They measure 0.4 to 0.6 $\mu$ in width by 4 to 8 $\mu$ in length (Weinberg and Séguin[6]) and may form filaments in old cultures. The organisms occur singly, occasionally form pairs and chains. In Adamson's[7] isolations, filaments were formed abundantly, the organisms often appearing as a tangled mass of fine elements. Bacilli smaller in alkaline agar.

**Spores** formed rapidly, large, oval, terminal. When attached to the organism they may appear spherical or oval and retain the stain well (Henry). Fully developed spores easily decolorized. Spores produced readily in alkaline agar, alkaline potato, ascitic fluid agar, brain broth, egg broth, and muscle broth. Spores always wider and often longer than the vegetative rods. Robertson described spores at both ends of the rods in her isolation. Von Hibler finds that the spores are destroyed by heating to 97° to 98° C. for eight minutes. Spores formed in the tissue as well as in cultures (Weinberg and Séguin).

**Motility** usually feeble, sometimes not demonstrable. Cilia stained by v. Hibler.

**Stains** irregularly. Gram-positive in young cultures. Gram-negative after three days, especially in fluid media.

Fig. 175.—*Bacillus tertius* from thirteen-day culture in dextrose agar, stained with carbol-fuchsin.

**Cultivated** easily, not requiring strongly anaërobic condition.

*Plain Agar.*—Colonies grow slowly, forming small, almost transparent surface films, greenish blue or iridescent under a hand-lens. Not raised except in the center of old colonies. Margins irregular or finely crenated, with no radiations or outgrowths. Colonies 2 mm. in diameter. No larger. Older colonies tend to become opaque. According to Henry the colonies may be opaquely granular from fine roots sent down into the depths of the agar.

*Agar Slant.*—Continuous surface film.

*Agar shake colonies* small, lenticular or irregular, without branching filaments (M. R. C.[8]).

*Deep agar* colonies minute, compact, opaque, lenticular masses (Hall[9]).

*Alkaline agar* colonies smaller than in plain agar. Growth scanty.

*Surface blood agar* colonies hemolytic and resemble dew-drops in transparency and shape when young (Hall). Colonies more opaque on prolonged cultivation.

*Glucose agar* colonies larger than on plain agar but almost transparent and very moist. They may show a less transparent center with a narrow marginal zone, regular or finely crenated (Adamson).

*Glucose Agar Stab.*—Growth along line. No penetration of the medium. Agar fragmented by gas formation in thirty-six to forty-eight hours.

*Neutral Red Glucose Agar.*—Gas production with a bright green fluorescence.

*Alkaline Egg Agar* (Weinberg and Séguin).—Surface colonies appear in two to four days and attain their maximum development in five to six days. They are either small, fine, almost transparent under low power, showing clear centers and irregular margins, or larger, opaque colonies with crenated or irregular edges. From those latter colonies irregular roots may extend to the depths of the medium.

*Gelatin.*—Arborescent colonies. No liquefaction.

*Inspissated Serum.*—Colonies isolated or confluent and tend to sink into the medium but cause no real liquefaction. In Adamson's isolation the colonies remained superficial without erosion of the medium.

*Alkaline Egg.*—Coagulation, the coagulum slowly sinking. No digestion.

*Dorset's Egg Medium.*—Scanty growth. No digestion or liquefaction. Small transparent surface colonies or fine surface film (Adamson).

*Alkaline Meat Medium.*—Acid and gas production in twenty-four hours. Not so marked as with *Bacillus Welchii*. The medium clears and the meat takes on a bright rose tint. Later the upper part of the meat is decolorized. No blackening. No digestion. No odor. According to Henry a pigment is formed which is an acid derivative of hemoglobin.

*Brain Medium.*—Some gas. Pink color. Very slight acid.

*Plain Broth.*—Good growth in twenty-four hours with a uniform turbidity which settles to the bottom of the tube in four to five days.

*Glucose Broth.*—Active fermentation with acid and gas production.

*Broth Containing Coagulated White of Egg.*—No digestion even after four to five weeks.

*Egg Broth.*—Soft clot. No discoloration. Gas. No digestion or shrinking of clot.

*Milk.*—Slow coagulation with the production of a friable clot in forty-eight hours. Gas development marked, the clot riddled or fractured with gas bubbles (Weinberg and Séguin). No digestion. Hall states that the clot in milk never becomes gas-blown as with *Bacillus Welchii*. Fermentation not stormy.

**Saccharolytic properties** marked. Fermentation of glucose, levulose, galactose, saccharose, maltose, lactose, mannose, xylose, starch, dextrin, glycogen, salicin, amygdalin, and mannite, with no action on raffinose, rhamnose, arabinose, inulin, saponin, dulcite, erythrite, sorbite, glycerin (Henry). The Medical Research Committee report failure to ferment glycerin, inulin, and dulcite. In Adamson's strains mannite was not fermented, one strain never attacked saccharose and two others first fermented it, then later lost this power. Gas composed of hygrogen and carbon dioxid. No action on cellulose.

**Proteolytic properties** lacking. No indol produced.

**Non-pathogenic.**

1. Fleming: Lancet, 1915, ii, 376.
2. v. Hibler: Untersuchungen über die pathogenen Anaëroben. Jena, 1908.
3. Rodella: Ztschr. f. Hyg., 1902, xxxix, 201.
4. Robertson: J. Path. and Bacteriol., 1915–16, xx, 327.
5. Henry: J. Path. and Bacteriol., 1916–17, xxi, 344.
6. Weinberg and Séguin: La gangrène gazeuse. 1916, p. 124.
7. Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.
8. Rep. Brit. Med. Research Com. No. 39.
9. Hall: J. Infect. Dis., 1922, xxx, 445.

### CLOSTRIDIUM COCHLEARIUM (McIntosh and Fildes)

Described apparently first by McIntosh and Fildes[1] as Type III C and by Douglas, Fleming, and Colebrook. Named by the British Medical Research Committee.[2] Identical with Adamson's Type III.[3] Frequently found in wounds both in early cases and in late septic infections.

**Morphology.**—Slender, actively motile rods, straight, usually isolated, rarely in pairs and chains. Gram-negative or weakly Gram-positive. Spores appear in forty-eight hours in cultures. They are large, almost spherical when young, oval when mature, terminal, giving the organism a spoon-shaped appearance after which it is named. Spores not readily formed on ordinary media. Best seen in alkaline digest broth containing fresh tissue (M. R. C.). Adamson reports rapid sporulation in ordinary broth, slower in glucose broth.

**Cultivated** under anaërobic conditions. Growth of pure cultures not vigorous. When mixed with *Bacillus sporogenes* the growth is more vigorous and sporulation active. Such a culture resembles *Bacillus putrificus*.

Surface colonies on *serum agar plates* or *serum agar slants* delicate glass-clear droplets, sometimes with faintly crenated edges (M. R. C.). Old colonies have opaque centers and marginal translucent or almost transparent zones (Adamson). Colonies in agar shakes lenticular. No offshoots.

*Agar Slant.*—Almost transparent film (Adamson).

*Glucose Agar.*—Growth slower than on plain agar. Colonies swollen, almost transparent or translucent.

*Deep Glucose Agar Stab.*—No gas. Translucent line growth. No radiation or offshoots into the media.

*Dorset's Egg Medium.*—Small, colorless, superficial, streptococcus-like colonies. Colonies may coalesce to form a surface film. Erosion of the surface may occur and a slight darkening. No digestion.

*Meat Medium.*—Slight reddening but no digestion, and very little gas. Characteristic non-putrid odor.

*Gelatin.*—No liquefaction according to the Medical Research Committee. Slow liquefaction and clearing according to Adamson.

*Inspissated Serum.*—Scanty growth. Fine, transparent film. No liquefaction.

*Broth.*—Turbidity, later precipitation.

*Glucose Broth.*—Turbidity greater than in ordinary broth with a more rapid settling. Growth most profuse in slightly acid or neutral broth, less profuse in alkaline broth. No indol.

*White of Egg Broth.*—Partial coagulation. No digestion.

*Milk.*—No acid. No coagulation. Small, white precipitate of casein. No digestion. (Reaction like that with *Bacillus tetani.*)

**No Saccharolytic Action.**—No acid or gas from sugars, starch, etc.

**Proteolytic action** very slight. Gelatin may be slowly liquefied. No liquefaction of coagulated serum, egg-meat, etc.

**Non-pathogenic.**

1. McIntosh and Fildes: Rep. Brit. Med. Research Com., 1917, No. 12.
2. Rep. Brit. Med. Research Com., 1919, No. 39.
3. Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.

### CLOSTRIDIUM S (Adamson)

Isolated by Adamson from 2 cases of war wounds and once from the heart's blood at autopsy. Named *Bacillus* S.

Like McIntosh and Fildes' Type III C (*Bacillus cochlearius*), but not the same.

**Morphology.**—Moderately thick, motile bacillus with round ends from neutral agar, occurring as short or long forms. Frequently curved. Arranged singly, in pairs, or short chains, or filaments often twisted or coiled. Gram-positive in young cultures, Gram-negative in cultures two to three days old. Spores formed rarely, not thicker than the organism, oval, elongated, or rectangular with parallel sides, terminal, or subterminal. On alkaline agar the bacilli are shorter, less frequently in chains or filaments and produce spores more readily than on ordinary agar. On glucose agar few spores are found but many involution forms. On inspissated serum the bacilli are slender and no spores are seen. Non-motile in broth, twisted and curved chains.

**Cultural Characters.**—*Agar colonies* circular, moist, translucent, whitish, or opaque. Thick in center with translucent margins. Edges smooth or indented. The organisms may grow over the surface as a translucent, continuous, moist film. Margins of colonies may coalesce forming a kind of thicker, more opaque island.

*Alkaline agar* growth less rapid. Colonies smaller, more translucent, less spreading.

*Glucose Agar.*—Colonies larger and thicker than on ordinary agar, but much the same. Colonies may also be regular, quite white, and opaque without marginal halo. Colonies apt to remain discrete.

*Deep Glucose Agar Stab.*—Line growth. No radiation or penetration of medium. Gas fragments the medium. With neutral red, bright green fluorescence develops and persists a long time.

*Dorset's Egg.*—Slow growth. Colonies small, colorless points, after four to five days conical and raised. Later they sink slightly into medium which becomes paler in color. A pale zone forms, a shallow saucer-like depression with a conical mass rising in middle. No other change in medium.

*Egg Broth.*—Coagulation. No digestion. No gas.

*White of Egg Broth.*—No digestion.

*Brain Medium.*—Gas. No digestion.

*Meat Medium.*—No digestion.

*Inspissated Serum.*—Colonies as minute points, also a very fine film. No liquefaction.

*Broth.*—Turbidity and clearing. Deposit. Acid broth gives more abundant growth than alkaline.

*Glucose Broth.*—Gas.

*Gelatin* liquefied. Later the bacilli are deposited at the bottom.
*Milk* reddened slightly. No digestion. No change in reaction. Slight precipitation of casein.
*Sugars.*—Slight acid with glucose in one strain. No change in two strains. Other sugars negative. No acid from starch.

Adamson: J. Path. and Bacteriol., 1918–19, xxii, 345.

### CLOSTRIDIUM SPERMOIDES (Ninni)

Isolated by Ninni from the soil as *Bacillus spermoides*. Slender rods 4 to 10 $\mu$ in length with terminal spores cylindrical, not round. Non-motile. Gram-positive. Cultivated anaërobically.

*Agar colonies* in twenty-four hours 1 mm. in diameter, grayish-white, transparent, humid and not viscous, margins smooth. Colonies develop to a size of 3 to 4 mm. Round punctiform colonies in the depths of *gelatin stab*, gradually extending along line of inoculation to the surface. Marked evolution of gas in twenty-four hours. After four to five days the gelatin is liquefied, the liquefaction not being characteristic. Complete liquefaction in about ten days. The liquid gelatin is limpid and shows a deposit of bacteria at the bottom. *Broth* turbid in twenty-four hours with a few bubbles of gas. Later the broth clears. Gas also in *liver broth*. Abundant gas formation in *glucose* and *lactose broth* with a highly acid reaction. *Milk* acidified and coagulated in twenty-four hours with a separation of limpid serum. Odor not disagreeable but like acetic or butyric acid. Acids partly lactic, partly volatile—acetic or higher acids.
No indol.
*Virulent* to guinea-pigs by subcutaneous or intraperitoneal inoculations of small doses, 0.2 c.c. liver broth cultures. The animals develop cachexia and die in five to seventeen days.

Ninni: Pathologica, 1920, xii, 385.

### CLOSTRIDIUM TYROSINOGENES (Hall and Finnerud)

Described by Hall and Finnerud as producing tyrosin crystals from ground meat, brain, salmon, milk and suspended casein medium not containing fermentable carbohydrates, *i. e.*, monosaccharids, in excess. Clouding of the media and vigorous gas production seen in early stages. Crystals appear in four to five days at 37° C. along with vigorous liquefaction of the protein and odorous evidence of putrefaction. The meat and brain media are blackened by $H_2S$ and iron freed by proteolysis from protein. Milk, salmon and casein not blackened except when an iron nail is added, even when $H_2S$ is present. Crystals of tyrosin obtained pure. They are soluble in boiling water, N/10 ammonia and dilute mineral acids, slightly soluble in dilute acetic acid, cold and hot absolute alcohol, ether, acetone, benzene, carbon disulphid, glycerin and chloroform. They give Pirie's, Hoffmann's, and Denige's tests for tyrosin.

The only other putrefactive organisms thus far described producing tyrosin are *Bacillus bifermentans*, *B. centrosporogenes*, and *B. histolyticus*. Tyrosin not produced by *B. Welchii*, *B. Novyi*, *B. butyricus*, *B. sporogenes*, *B. tertius*, *B. tetanomorphus*, *B. botulinus* A and B, *Vibrion septique*, *B. tetani*, *B. putrificus*, *B. chauvei*, and *B. sphenoides*.

Hall and Finnerud: Proc. Soc. Exper. Biol. and Med., 1921, xix, 48.

## GENUS 3. THERMOBACILLUS Jensen

Non-pigmented rods growing at high temperatures, 50° to 80° C. either as obligate thermophils requiring those temperatures, or as facultative thermophils growing both at ordinary and at high temperatures. Usually produce spores. Aërobic and anaërobic. Cultivated with some difficulty, growing only on certain media. Common in the environment. Usually without pathogenic properties. While these organisms should probably all be designated Thermobacillus, the names by which they were described may be retained for the present.

*Aërobic*

*Type species Bacillus thermophilus* Frankland.
" " No. I Rabinovitch.
" " No. II "
" " No. III "
" " No. IV "
" " No. V "
" " No. VI "
" " No. VII "
" " No. VIII "
" *ilidgensis capsulatus* Karlinsly.
*Bacterium Ludwigi* Karlinsky.
*Bacillus cylindricus* Blau.
" *robustus* "
" *tostus* "
" *callidus* "
" *thermophilus Jivoini* Georgevitch.
" " *Losanitchi* "

*Anaërobic*

*Clostridium thermoputrificum* Damon and Feirer.
" *thermoaërogenes* " "
" *thermoacidophilus* " "
" *thermochainus* " "

### BACILLUS THERMOPHILUS Frankland

Discovered by Miquel[1] in water of the Seine, in sewage and in the atmosphere, and described as living at 70° C.

First correctly named by Frankland.[2]

Same as *Bacillus thermophilus Miquelii* of Kruse,[3] *Bacterium thermophilum* (Miquel) of Migula,[4] and *Bacterium Miquelii* (Kruse) of Chester.[5]

**Morphology.**—Non-motile threads about 1 $\mu$ long and of various lengths.

**Spore-formation.**—Terminal refractile spores.

**Cultural Characters.**—Develops slowly at temperatures between 42° and 72° C. Does not grow on gelatin at 22° to 23° C. No growth on agar at 40° C. At 42° to 45° C. develops on slant agar as a white meniscus, spreading over the surface. Develops well at 50° to 65° C. In broth at 50° C. produces a turbidity and a fragile scum.

**Non-pathogenic.**

1. Miquel: Ann. de Microg., 1888, i, 4.
2. Frankland: Microörganisms of Water. 1894, p. 488.
3. Kruse: *In:* Die Mikroorganismen (Flügge). 1896, ii, 269.
4. Migula: System der Bakterien, 1900, ii, p. 342.
5. Chester: Manual of Determinative Bacteriology. 1914, p. 186.

### BACILLUS THERMOPHILUS No. I Rabinovitch

First isolated by Rabinovitch[1] from soil, snow, milk, maize, oats, barley, and from the intestinal contents of many animals, including mice, guinea-pigs, dogs, cows, goats, horses, pigeons, hens, parrots, ducks, fish, frogs, and from man.

Also known as *Bacterium Lydiæ* Migula.[2]

**Morphology.**—Non-motile rods often growing out into long threads and forming terminal oval spores.

**Resistance.**—Spores resist streaming steam five to six hours and drying four to five months.

**Cultural Characters.**—Develops best aërobically at temperatures between 60° and 70° C. Grows also anaërobically between 33° and 44° C. Develops on neutral and acid agar, best on slightly alkaline agar. Grows also on sugar agar and Pfeiffer's blood agar. No growth at 33° to 42° C. Moderate growth at 54° C. on agar (3 per cent.).

*Agar Plates.*—Symmetrical, coarsely granular colonies with irregular edges at 62° C.

*Potato.*—White colonies often crater-like.

*Broth.*—Rapid development with turbidity and flocculent sediment. Acid production.

*Litmus Milk.*—Acid production.

*Nitrates* reduced to nitrites.

**Non-pathogenic** to mice and pigeons.

1. Rabinovitch: Ztschr. f. Hyg., 1895, xx, 156.
2. Migula: System der Bakterien. 1900, ii, p. 343.

### BACILLUS THERMOPHILUS No. II Rabinovitch

Obtained by Rabinovitch from soil, snow, barley, milk, and from the intestinal contents of horses, beef, cows, coats, dogs, fish, frogs, pigeons, hens, parrots, and ducks.

Known also as *Bacterium viridulum* Migula.

**Morphology.**—Non-motile, medium-sized, slender bacilli with round, central spores, often somewhat curved, round, or bent. Irregular or granular when stained.

**Resistance.**—Spores resist streaming steam five to six hours and drying four to five months.

**Cultural Characters.**—No growth on agar at 33° to 39° C. Slight development at 42° C. Good growth on agar at 54° C. Slight development on potato at 54° C. Grows best at 62° C. Good anaërobic growth at 33° C.

*Agar Plates.*—Grayish-green, somewhat granular colonies gradually spreading with fine outgrowths.

*Potato.*—Grayish-yellow colonies with irregular edges.

*Broth.*—Alkali production.

**Non-pathogenic** to mice and pigeons.

### BACILLUS THERMOPHILUS No. III Rabinovitch

First found by Rabinovitch[1] in soil, milk, oats, corn, and in the intestinal contents of mice, guinea-pigs, fish, and man.

Known also as *Bacterium streptococciforme* Migula.

**Morphology.**—Moderately thick, non-motile rods with terminal oval spores.

**Resistance.**—Spores resist steam five to six hours and drying four to five months.

**Cultural Characters.**—Grows best at 60° to 70° C., also anaërobically at 33° C. No aërobic growth on agar at 33° C. Slight growth on agar at 39° C. No growth on potato. Abundant development on agar at 42° C., no growth on potato. Abundant on agar at 54° and 62.5° C.

*Agar Plates.*—Small, round, grayish-white colonies with sharply defined edges at 62.5° C., much resembling streptococcus colonies.

*Potato.*—Brown colonies at 62.5° C.

*Broth.*—Acid production in forty-eight hours.

**Non-pathogenic** to mice and pigeons.

### BACILLUS THERMOPHILUS No. IV Rabinovitch

Isolated by Rabinovitch from soil and from the dejecta of rabbits, pigeons, hens, parrots, ducks, beef, and man.

Known also as *Bacterium subthermophilum* Migula.

**Morphology.**—Non-motile rods often growing out into long threads with central round spores.

**Resistance.**—Spores resist streaming steam five to six hours and drying in the oven four to five months.

**Cultural Characters.**—Rapid development. Grows on agar at temperatures from 33° to 62.5° C. No growth on potato at 33° to 54° C. Good growth at 62.5° C.

*Agar Plates.*—Regular colorless colonies with many thin outgrowths.

*Potato.*—Red colonies coalescing to form a homogeneous expansion.

**Non-pathogenic** to mice and pigeons.

### Bacillus thermophilus No. V Rabinovitch

First found by Rabinovitch in barley and in the dejecta of guinea-pigs, rabbits, and frogs. Known also as *Bacterium intactum* Migula.

**Morphology.**—Thick, non-motile rods with terminal oval spores, often growing out into long threads.
**Resistance.**—Spores resist streaming steam five to six hours and drying four to five months.
**Grows** best at 62.5° C.
*Agar Plates.*—Colorless colonies like drops of water with granular centers at 62.5° C.
*Potato.*—Scanty development as a grayish-brown film at 62.5° C.
*Broth.*—A trace of acd production.
**Non-pathogenic** to mice and pigeons.

### Bacillus thermophilus No. VI Rabinovitch

Cultivated by Rabinovitch from the dejecta of mice, pigeons, hens, parrots, ducks, and beef. Known also as *Bacterium glaucescens* Migula.

**Morphology.**—Non-motile rods with terminal oval spores.
**Resistance.**—Spores resist streaming steam five to six hours and drying four to five months.
**Cultural Characters.**—Grows best at 62.5° C.
*Agar Plates.*—Grayish-green colonies coarsely granular in the centers with light, water-clear margins, at 62.5° C.
*Potato.*—Grayish colonies forming a moist expansion layer at 62.5° C.
*Broth.*—Abundant alkali production.
**Non-pathogenic** to mice and pigeons.

### Bacillus thermophilus No. VII Rabinovitch

Found by Rabinovitch in the excrement of mice, dogs, and cows. Known also as *Bacterium subdenticulatum* Migula.

**Morphology.**—Non-motile rods with oval terminal spores forming threads.
**Resistance.**—Spores resist streaming steam five to six hours and drying four to five months.
**Cultural Characters.**—Grows best at 62.5° C.
*Agar Plates.*—Regular coarsely granular colonies with irregular edges at 62.5° C.
*Potato.*—Grayish-white colonies at 62.5° C.
*Broth.*—Abundant alkali production.
**Non-pathogenic** to mice and pigeons.

### Bacillus thermophilus No. VIII Rabinovitch

Obtained by Rabinovitch from barley and from the excrement of doves, hens, parrots, and ducks. Known also as *Bacterium aqueum* Migula.

**Morphology.**—Non-motile rods with oval terminal spores.
**Resistance.**—Spores resist streaming steam five to six hours and drying four to five months.
**Cultural Characters.**—Grows best at 62.5° C.
*Agar Plates.*—Round, water-clear colonies, moderately granular, with sharply defined edges.
*Potato.*—Moist, grayish-brown colonies.
*Broth.*—Forms a trace of acid.
**Non-pathogenic** to mice and pigeons.

### Bacillus ilidzensis capsulatus (Karlinski)

Isolated originally from the water of the hot springs at Ilidze in Bosnia by Karlinski and named by him *Bacillus ilidzensis capsulatus*. Known also as *Bacterium ilidzensis* (Karlinski) Migula.

**Morphology.**—Thin, fairly long rods surrounded by definite capsules. Club-like involution forms on sheep blood-serum.

**Staining Reactions.**—Stains with the usual dyes. Gram-negative.

**Spore-formation.**—On potato not rendered alkaline, and on alkali albuminates at 68° C. the organism forms central, highly refractile spores.

**Resistance.**—The spores resist the action of streaming steam at 100° C. for four minutes. They survive over a month at a temperature of 51° C.

**Cultural Characters.**—No development below 50° C. Most favorable temperatures between 50° and 60° C. Between 60° and 70° C. the organisms develop, but more slowly.

*Agar Plates* (3 Per Cent. Agar).—Dull white, round colonies with clearly marked concentric layers in twenty-four hours.

*Agar Slant.*—Rapid growth over entire surface.

*Agar Stab.*—Little development along line of inoculation. Abundant surface growth.

*Alkali Albuminate.*—Dull white colonies with dome-like formation.

*Alkali Albuminate Slants.*—Growth limited to line of inoculation.

*Potato.*—Round, flat, snow-white, porcelain-like refractile colonies.

*Potato Streak.*—Shows development limited to line of inoculation.

*Sheep Blood-serum.*—Slight development as a very soft layer.

*Broth.*—Rapid formation of a turbidity with a thick surface scum.

Karlinski: Hyg. Rundschau, 1895, v, 685.

### BACTERIUM LUDWIGI Karlinski

Originally obtained by Karlinski from the water of the hot springs at Ilidze in Bosnia and named by him *Bacterium Ludwigi.*

**Morphology.**—Thick, short, non-motile rods.

**Spore-formation.**—No spores observed by Karlinski.

**Cultural Characters.**—No growth at temperature under 50° C. Grows abundantly at 50° to 52° C. Dies out at room temperature in twenty-four hours. Survives twenty-four hours at temperatures of 60° to 70° C., but dies out at 80° C. No anaërobic growth. Optimum temperature 55° to 57° C. Grows best on alkaline media. Survives unlimited time in filtered spring water at 50° C.

*Agar Plates* (3 Per Cent. Agar).—Citron-yellow, almost round, finely marked colonies which have a diameter of 3 mm. in three days.

*Agar Slant.*—Soft citron-yellow expansion.

*Agar Stab.*—Abundant superficial development. Slight growth along line of inoculation.

*Alkali Albuminate.*—Colonies irregularly indented, dark yellow, iridescent like ivy leaves.

*Alkali Albuminate Slant.*—Dark yellow, soft expansion.

*Clotted Sheep Blood-serum.*—Citron-yellow, soft expansion.

*Clotted Hen's Blood-serum.*—Slight, light yellow growth.

*Potato.*—Soft, light yellow, round colonies well developed in forty-eight hours.

*Potato Slant.*—Soft, light yellow expansion limited to line of inoculation.

*Broth.*—Yellow precipitate.

*Peptone* (1 Per Cent.).—Slight turbidity.

*Rice-water.*—No growth.

*Litmus Milk.*—Moderate acid production.

**Non-pathogenic.**

**Karlinski's Alkali Albuminate.**—Fresh hen's eggs preserved under 20 per cent. potassium hydroxid for fourteen days. The white of the eggs removed under aseptic precaution, cut into layers 2 to 4 mm. thick, and put into sterile plates. Further sterilization not necessary, since only about 1 per cent. are contaminated.

Karlinski: Hyg. Rundschau, 1895, v, 685.

### BACILLUS CYLINDRICUS Blau

Described by Blau from dry field earth near Marburg.

Homogeneous single and double rods after six to seven hours on dextrose agar, $\frac{1}{3}$ to 1 $\mu$ long, 0.9 to 1.2 $\mu$ broad. Later 1 to $1\frac{1}{2}$ $\mu$ long. Sporangia 0.8 to 1.1 $\mu$ in breadth by 4.5 to 7.5 $\mu$ in length, straight or slightly curved. Protoplasm homogeneous.

*Stains* with methylene-blue and fuchsin.

*Spores* polar, cylindrical to rod-shaped, with rounded ends, measuring 0.7 to 1.1 $\mu$ in breadth by 1.8 to 3 $\mu$ in length. Destroyed at 100° C. in nineteen to twenty hours.

**Motility** active due to long peritrichic flagella.

Glycogen stored in cells as reserve material.
Optimum temperature 60° to 70° C.

**Culture.**—Good growth on *dextrose agar* at 60° C. White or transparent colonies after ten hours, the size of pin-heads. After twenty hours these colonies coalesce to a coherent mass covering the entire agar surface. This mass is white to yellowish-gray in color, glistening, homogeneous, or finely granular. Condensation water turbid. After thirty hours' growth yellowish, later yellowish-brown.

*Dextrose Agar Stab.*—After twenty-four hours yellowish-gray, thin colony at point of puncture. Later this surface colony increases in thickness and the growth penetrates the medium to a depth of about 4 mm.

*Potato.*—No growth.

*Carrots.*—No growth.

Blau: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xv, 97.

### Bacillus robustus Blau

Isolated by Blau[1] at a temperature of 60° C. from soil near Marburg.

After three and a half hours on dextrose agar rods 1 to 2 $\mu$ long, 1 to 2 $\mu$ broad. Sporangia 1.1 to 1.3 $\mu$ in breadth, 3.3 to 3.5 $\mu$ in length.

**Spores** central to terminal, cylindrical, oval, and round, 0.9 to 1 $\mu$ in breadth, 1.6 to 2.2 $\mu$ in length.

**Motility** active. Flagella peritrichic, demonstrated by acid violet.

Glycogen stored in cells as reserve material.

Optimum temperature 55° to 60° C.

Colonies on *dextrose agar*, six to seven hours, transparent, yellowish-white streaks. After fourteen hours colonies wider, yellowish-white, homogeneous with glistening surface. Water of condensation turbid. After two to three days colonies whitish-gray, dry, less glistening. Colonies finely granular.

*Dextrose Agar Stab.*—After twenty-four hours thick, white surface colony penetrating line of stab 2 mm. After forty-eight hours line growth in stab to a depth of 1.3 cm. with short branches.

*Potato.*—Punctiform, whitish growth after twenty-four hours.

Odor like sour milk.

*Carrots.*—Very delicate, transparent growth.

No gas formation.

Blau: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xv, 97.

### Bacillus tostus Blau

Recovered by Blau from soil in Berlin.

Vegetative rods after three and a half hours on dextrose agar 1.2 to 1.3 $\mu$ in breadth by 1 to 2 $\mu$ in length, straight or slightly curved. Homogeneous, arranged in single cells, double cells, and threads of 3 to 6 cells. Also rods 2 to 3 $\mu$ in length. Sporangia vary from 1.1 to 3.1 $\mu$ in width and 1.5 to 7 to 8 $\mu$ in length. Spores oval, elliptical and round, terminal, 0.8 to 1.6 $\mu$ in width, 1.5 to 2.2 $\mu$ in length.

**Destroyed** at 100° C. in nineteen to twenty hours.

**Motility.**—Peritrichic flagella demonstrated by acid violet.

Glycogen stored in cells as reserve material.

Optimum temperature between 60° and 70° C.

Grows best on slightly alkaline agar.

*Dextrose Agar Plates.*—At 60° C. after ten to eleven hours small, white, or whitish-yellow colonies the size of pin-heads, medium thickness, almost circular, edges jagged. Also thin colonies which appear veil-like microscopically and finely granular under the microscope. Peculiar unpleasant odor. After forty-eight hours colonies somewhat larger, with pointed processes on the edges with broad bases. Colonies white to porcelain-like. Later the colonies are larger and the disagreeable odor more marked.

*Dextrose Agar Slant.*—After five to five and a half hours fine, milk-like, grayish-white, broad streak. After nine hours whitish-gray, less transparent. After fourteen hours broad thin, white-gray to yellow-gray streak with smooth edges, showing various small lobulations. Surface smooth and glistening. Soft in consistence. Water of condensation turbid. Later the growth is grayish-white to yellowish-white, porcelain-like. Water of condensation milky.

*Dextrose Agar Stab.*—After twenty-four hours whitish-yellow, moist, glistening expansion covering entire surface with a thread-like growth 5 mm. deep in line of inoculation. In

three days the line growth is 1½ cm. long with short hair-like processes. After fourteen days line growth 2 cm. deep with hair-like lateral processes in upper half. Later surface expansion grayish-yellow.

*Potato.*—No growth.

*Carrots.*—Small punctiform colonies which fail to develop.

No gas formation.

Blau: Centralbl. f. Bakteriol. [etc.], 2 Abt., 1906, xv, 97.

### Bacillus callidus Blau

Obtained by Blau from soil at Berlin.

Vegetative rods, five to six hours on dextrose agar, about 1 to 1½ μ long and 0.8 to 1 μ broad. Occur as single cells and chains of four to twelve individuals.

Sporangia 0.8 to 1 μ broad, 4.5 to 4.9 μ long.

Spores terminal, cylindrical, rod-shaped, or oval, 0.6 to 1.2 μ broad, 1.5 to 2.1 μ long.

Spores destroyed at 100° C. in seven and a half to eight hours.

**Motility** undulatory. Long peritrichic flagella demonstrated by acid violet.

Volutin and glycogen stored in cells as reserve material.

Optimum temperature 60° to 65° C.

*Dextrose Agar Colonies.*—After fourteen to fifteen hours whitish-gray, round colonies 1 to 3 mm. in diameter. The edges show irregular prolongations. Microscopically the centers are finely granular, the peripheries showing wavy threads. After twenty-four hours the colonies are larger, their prolongations have characteristic frosty appearance. Color whitish-gray like that of panes of glass. The culture has a peculiar sour, lime-like odor.

*Dextrose Agar Slant.*—After ten to eleven hours fine, delicate, vapor-like streak, colorless, transparent. After sixteen hours thin yellow-gray to white-gray growth over whole surface with frost-like extensions in the upper part. Water of condensation slightly turbid. After twenty-four hours entire surface covered with growth, a fine delicate precipitate in water of condensation. Later the growth is thin, yellowish-white or whitish-gray, with turbidity and floccules in water of condensation.

*Dextrose Agar Stab.*—Yellowish-white expansion covering entire surface, line growth 2 mm. in depth, without prolongations. No change in two to three weeks. No branches.

*Potato.*—No growth.

*Carrots.*—No growth.

No gas formation.

Blau: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1906, xv, 97.

### Bacillus thermophilus Jivoini Georgevitch

Cultivated by Georgevitch from warm springs in Serbia having a temperature of 50½° C.

**Morphology.**—Short rods from 5 to 7.5 μ in length and 2 μ in thickness, with rounded ends, arranged as single cells or as chains. Protoplasm within the cell granular. Stains well in Loeffler's methylene-blue and gentian-violet, poorly in fuchsin. When stained with Ziehl's fuchsin or Loeffler's methylene-blue the protoplasmic granules are unstained. They may be stained by boiling in fuchsin and are acid-fast, resisting decolorization with 5 per cent. sulphuric acid. Regarded by Georgevitch as the same as Bunge's acid-fast particles. The organisms are surrounded by a capsule which may be demonstrated by staining the bacillus with 2 per cent. gentian-violet and decolorizing with 1 per cent. acetic acid. Gram-positive. Spores central or slightly excentric, round, or elliptical.

**Cultivated** as an outspoken aërobe easily on 2 per cent. agar, gelatin, potato, and in bouillon at 42° C. Grows well at 49° C., but not at 50° C. Optimum temperature 43° to 45° C.

**Cultural Characters.**—*Agar 2 Per Cent.*—After fourteen hours at 42° C. colonies circular, gray, later acquiring a light color-tone and porcelain lustre. Superficial colonies have raised lighter edges, darker centers.

*Potato.*—After fifteen hours a white, soft, pale expansion, which covers the entire surface.

*Gelatin.*—Deep colonies after forty-eight hours star-shaped, 2 to 3 mm. in size, with compact centers and 4 to 5 thick radiations. Under low power these radiations are found to consist of a number of very long, straight, or slightly wavy threads. Surface colonies are circular with fringed edges.

*Broth.*—Rapid growth at 41° C. After fourteen hours the culture contains short actively motile individuals. At 43° C. after fifteen hours the broth is turbid and contains very long rods.

No acid or gas in glucose or milk.

Regarded by the author as closely related to *Bacillus illidzensis capsulatus* of Karlinski which grows, however, at 60° to 70° C.

Georgevitch: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1910, xxvii, 150.

### Bacillus thermophilus Losanitchi Georgevitch

Cultivated by Georgevitch from hot sulphur springs in Serbia with a temperature of 83° C. An obligate thermophil.

**Morphology.**—Rods with rounded ends 1 by 3 $\mu$ in size, arranged as single individuals, and shorter and longer chains. No capsule. Spores formed in centers of the rods at 51° C., oval, $\frac{1}{2}$ by 1 $\mu$ in size.

**Staining Reactions.**—Gram-positive.

**Motility.**—Young individuals from agar cultures slowly motile. From bouillon motility more active.

**Cultivation.**—Cultivated as an outspoken aërobe easily on 2 per cent. agar with the addition of $\frac{1}{2}$ to 1 c.c. of the original sulphur water or of its sulphur compounds. Grows also but not so well on ordinary agar. Grows only at 45° to 78° C. Optimum temperature 72° to 73° C.

*Agar 2 Per Cent.*—Colonies round, dark yellow, entire, at first soft and glistening, later dry, ash-gray, and skin-like. Deep colonies dentritic, browner. Superficial colonies grow by increasing radially at various points, forming finger-like projections. These finger-like prolongations coalesce and cover the whole surface of the agar.

*Agar Slant.*—Dark yellow layer covering entire surface.

*Potato.*—Good growth. Expansion first whitish and glistening, later dry, granular, and dirty-white.

No growth on *gelatin* at 22° C.

No gas or acid in *glucose* solution or *milk*.

*Broth.*—After fourteen hours a fairly hard, dirty-white scum.

Georgevitch: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1910, xxvii, 150.

### Clostridium thermoputrificum Damon and Feirer

An obligate anaërobic thermophile obtained by Damon and Feirer from fecal material derived from the horse. It undoubtedly represents a group of organisms having considerable putrefactive power and capable of exhibiting its reactions at 37° C. as well as at 55° C.

**Morphology.**—Uniform short rods with homogeneous protoplasm and rounded ends. The average dimensions of the rods taken from a forty-eight-hour chopped meat culture incubated at 55° C. are 5 by 2.5 $\mu$.

**Staining Properties.**—The organisms are Gram-positive in forty-eight-hour meat cultures. They lose their ability to retain the gentian-violet after five days' incubation.

**Motility.**—Non-motile in meat cultures examined every two hours from four to forty-eight hours' incubation. No flagella demonstrable.

**Spore-formation.**—Spores are observed in forty-eight-hour meat cultures. They are terminal and oval, and bulge the rod slightly.

**Cultivation.**—*Agar Shake Colonies.*—Single colonies in 2 per cent. veal infusion agar are small, discus shaped, with well-defined margins as seen under the lower power.

*Cooked Meat Medium.*—After two days' incubation at from 37° to 55° C. gas bubbles are noted throughout the medium At 37° C. the vaselin cap is raised about 1 cm. at the end of four days' incubation. Upon penetration of the vaselin cap a strong putrefactive odor is noted, and at the end of four days there is a definite proteolysis as indicated by the digestion of the meat. There is no change in the color of the medium.

*Sugar-free Cooked Meat.*—Shows reddening and a hazy diffuse growth throughout the medium.

In both media the broth above the meat remains clear except for a narrow zone of turbidity immediately above the meat particles.

**Fermentation Reaction.**—Lactose, glucose, starch. Growth at from 37° to 55° C., but no acid or gas produced.

Maltose, glycerol, sucrose, mannitol, and inulin. Growth with a marked production of gas, but no acid indicated at 37° to 55° C. with Andrade indicator.

*Lead Acetate Infusion Agar.*—Hydrogen sulphid produced as shown by a black coloration of the medium at 37° to 55° C.

This culture does not reduce nitrates; it has no action on gelatin, and does not produce indol.

Damon and Feirer: J. Bacteriol., 1925, x, 37.

### CLOSTRIDIUM THERMOAËROGENES Damon and Feirer

An obligate anaërobe growing only at 45° to 55° C., isolated by Damon and Feirer from horse manure. A number of cultures were obtained which gave identical reactions as indicated below. None of the cultures exhibited any putrefactive action. Gas, however, was produced in certain of the carbohydrate media, but no acid was formed.

**Morphology.**—A uniform medium-sized rod with granular protoplasm and rounded ends. The average dimensions of the rods in a forty-eight-hour meat culture incubated at 55° C. are 7 by 1.8 $\mu$.

**Staining Properties.**—Gram-negative in forty-eight-hour meat cultures.

**Motility.**—Non-motile in meat cultures examined every two hours after four to forty-eight hours. Incubation at 55° C. No flagella demonstrable.

**Spore-formation.**—Spores observed in forty-eight-hour meat cultures incubated at 60° C. They are terminal, oval, and bulge the rod slightly.

**Cultivation.**—*Agar Shake Colonies.*—Single colonies in 2 per cent. veal infusion agar are round and have well defined margins under the low power.

*Cooked Meat Medium.*—After two days' incubation at 55° C. gas bubbles are noted throughout the medium. There is no change in the color of the meat or sign of digestion.

*Sugar-free Cooked Meat.*—Shows a reddening and a hazy diffuse growth throughout the meat particles. A narrow zone of broth above the meat is turbid, above this the broth is clear.

**Fermentation Reactions.**—Lactose, glycerol, sucrose, mannitol, and glucose show growth, but no acid or gas is formed.

*Maltose and Inulin.*—Gas is produced, but no acid is indicated by the Andrade.

*Starch.*—Gas and acid produced.

*Nitrate Reduction.*—Nitrates are reduced to nitrites after forty-eight hours' incubation at 55° C. These cultures show no reaction on lead acetate or gelatin and do not produce indol.

One culture was isolated which was identical with *Clostridium thermoaërogenes* except that it produced acid in inulin.

Three cultures were isolated which were morphologically similar and exhibited the same reactions at 55° C. as the above organism, but also had the ability to grow at 37° C. without any alteration of the media.

### CLOSTRIDIUM THERMOACIDOPHILUS Damon and Feirer

An obligate anaërobic thermophile isolated by Damon and Feirer from horse manure. A number of cultures were obtained which gave acid on some carbohydrates and acid and gas on others. None of the cultures exhibited any digestion of the meat.

**Morphology.**—A long rod with granular protoplasm and rounded ends. The average dimensions of the rods in a forty-eight-hour meat culture incubated at 55° C. are 10.8 by 1.2 $\mu$.

**Staining Properties.**—Gram-positive in a forty-eight-hour meat culture. The organisms lose their ability to retain the gentian-violet after five days' incubation.

**Motility.**—Non-motile in meat cultures examined every two hours after four to forty-eight hours' incubation at 55° C. No flagella were demonstrable.

**Spore-formation.**—Spores observed in forty-eight-hour meat cultures incubated at 60° C. They are terminal, oval, and bulge the rod slightly.

**Cultivation.**—*Agar Shake Colonies.*—In 2 per cent. veal infusion agar shake tubes single colonies are like a burr; no definite margin could be seen under low power.

*Cooked Meat Medium.*—After two days' incubation at 55° C. gas bubbles are noted

throughout the medium. There is no change in color and no digestion is noticed in the meat.

*Sugar-free Meat Medium.*—Shows a reddening and a hazy diffuse growth throughout the meat particles. The broth is clear except for a small zone just adjacent to the meat.

**Fermentation Reactions.**—At 55° C. lactose, maltose, and glucose show growth, but no acid or gas is observed.

No gas is produced in glycerol, starch, and sucrose, but acid is observed. Gas and acid are seen in mannitol and inulin.

**Nitrate Reduction.**—Nitrates are reduced to nitrites in forty-eight hours.

These cultures show no reaction on lead acetate or gelatin, and do not produce indol.

One culture was isolated which was morphologically similar to the above strain and exhibited the same carbohydrate reactions at 55° C., but did not reduce nitrates.

Two cultures were isolated which were similar to *Clostridium thermoacidophilis*, but grew at 37° C. with no apparent reactions, although one of them has the power to liquefy gelatin at 55° C.

### CLOSTRIDIUM THERMOCHAINUS Damon and Feirer

An obligate anaërobic thermophil isolated by Damon and Feirer from horse feces. Three cultures were isolated which produced acid from starch and sucrose. None of the cultures exhibited any visible action on meat.

**Morphology.**—A uniform medium rod with granular protoplasm and slightly rounded ends. The rods may appear singly or in chains of from 3 to 6 organisms. The average dimensions of the single rods in a forty-eight-hour meat culture incubated at 55° C. are 7 by 1.8 $\mu$.

**Staining Properties.**—Gram-positive in a forty-eight-hour meat culture. After five days' incubation the organisms lose their ability to retain the gentian-violet.

**Motility.**—The cultures are non-motile in meat cultures examined every two hours after four to forty-eight hours' incubation at 55° C. No flagella are demonstrable.

**Spore-formation.**—Spores observed in forty-eight-hour meat cultures incubated at 60° C. They are terminal, oval and bulge the rod slightly.

**Colonies.**—*Agar Shake Colonies.*—In 2 per cent. veal-infusion agar single colonies are round with well-defined margins under low power magnification.

*Cooked Meat Media.*—After two days' incubation at 55° C. gas bubbles are noted throughout the medium. There is no change in color or digestion noticed in the meat.

*Sugar-free meat medium* shows a reddening and a hazy diffuse growth throughout the meat particles. The broth is clear above the meat.

**Fermentation Reactions.**—At 55° C. maltose, glycerol, lactose, mannitol, inulin, and glucose show growth, but no acid with Andrade indicator. No gas bubbles are seen in the medium.

Starch and sucrose show slight acid production with Andrade indicator, but no gas is observed.

*Nitrates* are reduced to nitrites after two days' incubation at 55° C. These cultures show no reaction on gelatin or on lead acetate, and do not produce indol.

# PART III

# DISTRIBUTION OF BACTERIA

## Chapter I

## PERSONAL BACTERIA

### BACTERIA OF THE CUTANEOUS SURFACES

THE exposed cutaneous surfaces of the body like the hands and face are constantly open to the deposition of bacteria from the air and dust and are not infrequently soiled by the secretion of the mouth and nose. Bacteria must also be deposited upon the skin of the hands and face from the soil, especially in workmen and in children playing in the dirt. Many different species must, therefore, be present on the exposed skin from time to time and we would expect a wide and varied flora there. Cultures reveal surprisingly few bacteria, however, and in general only non-pathogenic organisms. Pigmented bacteria and spore-bearing organisms are occasionally encountered. Evidently the ordinary species do not find proper conditions for the prolongation of their life on the skin surfaces and apparently do not survive there any length of time. The character of the epithelium is such as not to furnish a good foothold for bacteria, and we cannot speak of a normal flora of the skin as we speak of a normal flora of the mucous surfaces, like those of the alimentary canal and vagina.

Bacteria are always found in the deeper layers of the skin, in the ducts of the sebaceous glands and about the hair-follicles. The species are practically limited to white micrococci of low virulence, such as *Staphylococcus epidermidis albus* of Welch, which may have considerable importance in low-grade surgical infections such as stitch abscesses, and to diphtheroids like the acne bacillus of Gilchrist.

On the *hands* bacteria persist under the nails and about the junction of the nails and fingers where they are difficult to reach by disinfectants. Pathogenic organisms have been found in only a few instances. Pyogenic cocci may appear on the hands of individuals who come in direct contact with large suppurating wounds. Tubercle bacilli and typhoid bacilli have been isolated occasionally from the hands of attendants in institutions where patients with tuberculosis and typhoid fever are cared for. Despite the small number of instances in which pathogens have been obtained from the hands, epidemiological evidence suggests that in such diseases as typhoid fever the typhoid bacillus is present there from time to time. The frequency with which food and milk carry this disease, and the evidence that the contamination with *Bacillus typhosus* arises from the handling of these articles of diet by individuals who are discharging this organism in the feces and urine cannot be ignored. Evidently such organisms as the typhoid bacillus are occasionally deposited upon the hands, but do not survive there except for very brief periods. In this connection it is important to remember that the hands are frequently soiled with material containing *Bacillus coli* and other intestinal bacteria. Thus Winslow[1] has found this organism on the hands of students in Boston in 5 to 10 per cent. of the cases. Apparently the thorough and repeated cleansing of the hands with soap and water

removes the majority of bacteria which may be present there adventitiously. The frequency with which infections develop on the hands, especially about the nails, suggests that pathogenic organisms are present more often than is indicated by the results of bacteriological examination.

Bacteria must also be present frequently on the skin of the *face*. The lips are constantly soiled with secretions of the mouth and nose. It is doubtful, however, whether bacteria accustomed to the moisture and lack of light in the oral and nasal cavities would survive outside these cavities on the skin of the face. Certain types of infection are somewhat common on the face, especially the impetigo of children due to *Staphylococcus aureus*, and the sycosis of adults, also due to some type of staphylococcus. Infections about the lips with staphylococci and streptococci are by no means infrequent and often spread rapidly to other parts of the body. Similar infections are also somewhat common just at the external orifices of the anterior nares and about the hair-follicles of the nose. The skin of the external ear is often the site of staphylococcus infections, and a peculiar lesion occasionally develops in the external meatus due to an aspergillus, *Aspergillus niger* or *Aspergillus fumigatus*.

On the cutaneous surfaces of the body ordinarily covered by clothing the conditions are somewhat different from those on the exposed surfaces. The bacteria are protected from the action of light and air and the moist conditions there favor their survival. The skin of the buttocks is constantly soiled by the discharges of the intestine and must always harbor intestinal bacteria. There is probably also a fairly rich bacterial life in the folds of the skin about the groin and axilla and here *Bacillus pyocyaneus* is not infrequently present, as shown by the bluish-green stains on the underclothing. Certain types of bacteria are also constantly found on the cutaneous and mucous surfaces of the external genitalia in both men and women, especially acid-fast smegma bacilli, partially acid-fast diphtheroids, and spirochetes.

Bacteria are frequently present about the *feet*, especially under the toenails and between the toes, and here special organisms may be found able to decompose fats and fatty acids and produce disagreeable odors. In individuals with filthy habits the bacteria about the feet are naturally more abundant than in the cleanly. Indeed in some instances the spores of such pathogenes as the tetanus bacillus have been found in the dirt between and about the toes. Spreading infections starting from the toes and from the skin of other areas of the feet are by no means uncommon, chiefly due to staphylococci and streptococci.

## BACTERIA OF THE EYE

Despite the constant deposition of bacteria which must take place on the mucous membrane of the eye cultures reveal but few organisms, as a rule only such species as *Staphylococcus albus*. The bacteria are apparently removed mechanically by the secretions which pass down the lacrimal duct into the nose. Thus it has been shown that if pigmented organisms, such as *Bacillus prodigiosus*, be dropped on the normal conjunctiva they disappear from the eye and appear in the nose in a surprisingly short time. When the lacrimal duct is stopped up the secretions are dammed back into the eye and ocular infections frequently result.

Conjunctivitis or inflammation of the ocular and palpebral conjunctiva may be produced by a variety of organisms, the most important of which are the gonococcus, pneumococcus, and diplobacillus of Morax and Axenfeld. Inflammation of the deeper structures of the eye is rarely due to the tubercle bacillus.

## BACTERIA OF THE ANTERIOR NARES

Under normal conditions the nasal cavities are in constant communication with the air of the external world and one would expect a rich development of micro-organisms there. These cavities are also connected with other parts of the upper respiratory passages. The nasal mucosa acts as a kind of filter, however, and the air after passing it contains but a few bacteria. Thomson and Hewlett[2] found that air containing 29 fungus spores and 9 bacteria per cubic centimeter was germ-free after passing the nose. After spraying the nose with *Bacillus prodigiosus* these observers showed that the organism disappeared in two hours. Bloomfield[3] recently, Hildebrandt[4] also found that in normal breathing the micro-organisms in the air were all caught in the nasal mucosa. With excessive pollution of the atmosphere the nasal mucosa may fail to hold back all the organisms, since in rabbits fungus spores were found to pass clear into the lungs. Wright[5] has estimated that respiratory air with a velocity of 1 liter per minute (nine times slower than normal) loses three-quarters to four-fifths of its organisms in the nose. It is thus evident that bacteria are constantly being deposited on the nasal mucosa and we would expect to find a great many there. Cultures, however, reveal but few under normal conditions, the great mass of organisms being removed. Part of this removal is effected by the stream of mucus and the action of the ciliated epithelium, the current always being directed outward. It is possible also that some of the bacteria are ingested by phagocytes (leukocytes) and that they are destroyed in part by the nasal secretions. Viollet[6] believes that there is a nasal phagocytosis for streptococci and pneumococci, but not for the Friedländer bacillus. Klemperer[7] found that the nasal secretions were not bactericidal, although the ordinary organisms which grow well on artificial media grew badly on them. A bactericidal action of the nasal discharges upon the anthrax bacillus has been reported by Würtz and Lermoyez,[8] but this has not been confirmed. Park and Williams[9] have observed that the secretion of the nose has little bactericidal action on the diphtheria bacillus, streptococci, and staphylococci, but regard it as a poor medium for the development of bacteria. Gerber[10] believes that the secretions are a good medium for the leprosy bacillus, and Küster[11] claims that leprosy bacilli will develop in broth containing leprous nasal secretions. It is thus evident that under normal circumstances the bacteria deposited upon the mucosa of the nose are effectively disposed of, some being removed by the downward stream of mucus, some being destroyed by the secretions, some being removed by phagocytes, and some failing to grow because they do not find the proper conditions for development. The bacteria which may occasionally be isolated represent transitory inhabitants.

Various pathogenic organisms are encountered in the anterior nares from time to time. Thus von Besser[12] in an examination of 81 individuals found the pneumococcus six times, pyogenic streptococci five times, and *Staphylococcus aureus* seven times. He also found a certain number of spiral organisms. The most extensive investigations of the nasal mucosa are those of Neumann,[13] who examined over 200 individuals, of whom 111 were regarded as normal. The table on page 791 gives a synopsis of Neumann's findings.

In *infections* of the anterior nares various organisms may play an etiological rôle. The most important of these are such pathogenes as the pneumococci and streptococci, influenza bacilli, and Friedländer bacilli. The infections frequently extend into the accessory nasal passages like the antrum of Highmore and the ethmoidal sinuses. Crowe and Thacker-Neville[14] believe that the hemolytic streptococcus is the predominating organism in paransal sinus infections, next in frequency being the influenza bacillus and

the pneumococcus. When disease occurs in other parts of the respiratory passages pathogens may be encountered in the nasal secretions. Thus the meningococcus is present in meningitis and the diphtheria bacillus occasionally in diphtheria. Not infrequently in diphtheria the disease extends into the nose and a characteristic false membrane is formed there. In such cases virulent diphtheria bacilli are abundant in the nasal discharges.

| | Per cent. |
|---|---|
| Diphtheroids present in | 98–100 |
| Staphylococcus albus, | 98 |
| Staphylococcus aureus, | 30 |
| Staphylococcus citreus, | 12 |
| Sarcinæ, | 8 |
| Pigmented micrococci, | 8 |
| Micrococcus roseus, | 4 |
| Pneumococci, | 4 |
| Friedländer bacilli, | 6 |
| Lactis aërogenes, | 4 |
| Ozœna bacillus, | 3 |
| Bacillus coli, | 12 |
| Molds, | 20 |
| Yeasts, | 2 |
| Bacillus mesentericus, | 3 |

Apart from inflammations produced by these well-known organisms the etiology of the coryza known as the "common cold" is still obscure, and we are ignorant of the type of organism which is the responsible agent.

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## BACTERIA OF THE MOUTH

Under normal circumstances the buccal cavity offers very favorable conditions for the multiplication of bacteria, the saliva, mucus, dead epithelial cells, tooth pulp, and food particles all contributing to furnish a medium rich in substances which favor the growth of various types of micro-organisms. Physical conditions in the mouth are also conducive to the growth of bacteria. The temperature is almost uniformly maintained beneath the tongue at about 37° C., and this is suitable for human pathogens and for many which are not necessarily pathogenic, but which grow only on the body surfaces. Between the jaws and the cheeks and between the jaws and the lips the temperature varies and is often slightly lower. Here species may grow well whose optimum is below 37° C. The buccal cavity is dark and moist and bacteria are thus protected from destruction by light and drying.

The *saliva* is usually neutral or slightly alkaline, but may be acid in reaction. The reaction is not always the same. Thus it may be alkaline on moist portions of the tongue, acid in pockets of the mucous membrane. Over 99 per cent. of the saliva is water, the rest organic and inorganic salts (NaCl, KCl, SKCN), traces of protein, mucin, and the ferment ptyalin which converts starch to sugar and dextrin. Sanarelli[1] has shown that human saliva filtered free from organisms is a poor medium for the growth of bacteria and may even be slightly bactericidal. The total saliva changes its composition readily, is easily fermented, and furnishes an excellent culture material.

The *tooth pulp* is a favorable nidus for micro-organisms. The dentin is easily decalcified, especially by acids, and is then readily attacked by bacteria, with resulting caries. In the space between the tooth and the tooth flesh mouth organisms are frequently found in almost pure culture, particularly the mouth spirochetes, especially if a slight irritation has produced any excessive secretion. Small abscesses are very common about the roots of the teeth.

The *tonsils* are easily invaded by bacteria, especially by such organisms as streptococci, staphylococci, and pneumococci. Occasionally they are invaded by the tubercle bacillus either as a primary infection or as part of a generalized tuberculosis (MacCready and Crowe[2]).

*Bacteria Usually Found in the Mouth.*—A large number of different species of bacteria may be found occasionally in the buccal cavity, taken in with food and drink, and present there probably only for brief periods. Thus Miller,[3] who has made the most extensive investigations in this field, reported over 100 different varieties. It is very difficult to say which species are there regularly because of the constant intake of new material. Thus chromogenic cocci are sometimes cultivated from the mouth, but can hardly be regarded as regular inhabitants of it. Certain species may be found, however, in every mouth, and these may be regarded as the true mouth organisms. The majority of these cannot be cultivated or can be cultivated only with difficulty. The most important are the following:

1. Certain large bacteria staining red or blue with iodin. These may conveniently be grouped together as *Bacillus buccalis maximus*.

2. Spirochetes of various sizes and shapes, originally observed by Leeuwenhoek, Cohn, and Koch. Several of these have now been cultivated, but the classification is still very confused. They may conveniently be classified according to size as *Spirochæta dentium*, the large form, *Spirochæta buccalis*, the small form, and *Spirochæta media*, the form intermediate in size between these two.

3. Long unbranched threads known as leptothrix or leptotrichia. The most important are *Leptothrix buccalis* Vignol (possibly the same as *Leptothrix maxima* of Miller) or *Leptothrix buccalis* of Robin, *Leptothrix innominata* of Miller, and *Leptothrix racemosa* of Williams and Goodby.

4. Vibrios resembling the vibrio of Asiatic cholera. Some of these have been cultivated and are frequently known as Miller's vibrio or Miller's spirillum. The true vibrio or spirillum of Miller, however, has not been cultivated artificially. A similar species is known as *Spirillum sputigenum* of Miller.

5. Fusiform bacilli, usually found in association with the mouth spirochetes.

6. Certain large cocci containing substances staining red with iodin. The most important are *Iodococcus magnus*, *Iodococcus parvus*, and *Iodococcus vaginatus* of Miller.

In addition to these organisms, which may be regarded as the true mouth bacteria, many other species are found there occasionally in larger or smaller numbers. Among these the most important are staphylococci, streptococci, and pneumococci. Of the staphylococcus, both the "aureus" and "albus" are occasionally encountered, the latter more frequently than the former. Non-hemolytic streptococci, usually without virulence, are frequently present in the normal buccal cavity and rarely hemolytic streptococci of low virulence. Hemolytic streptococci of high virulence may also be present, but usually after disease of the upper respiratory passages. Pneumococci of low virulence are found in a large number of individuals, according to Zinsser[4] in about 30 per cent. of all cases. These pneumococci may produce inflammatory conditions in the lungs and bronchi, but the disease is usually of low grade and of short duration. Virulent pneumococci, capable of causing the more severe grades of lobar pneumonia, are present in the mouth somewhat infrequently, usually in convalescents or temporary carriers (Stillman[5]). *Micrococcus tetragenus* also is found occasionally in the normal mouth. Besides these species Gram-negative cocci are sometimes present, the most important being *Micrococcus catarrhalis*, *Micrococcus pharyngis siccus*, *Micrococcus flavus*, and the Gram-negative chromogens of Elser and Huntoon.[6] Occasionally the meningococcus is present, chiefly in convalescents or temporary carriers.

Various bacilli may rarely be encountered. Non-virulent diphtheria bacilli and closely related diphtheroids like Hofmann's bacillus are often present. Rarely organisms like the Friedländer bacillus are to be found.

Anaërobic bacteria are undoubtedly present in the buccal cavity under normal conditions, but few observations of value have been made upon them. Holman and Krock[7] have described small filter-passing cocci or bacilli and similar organisms have been occasionally seen by others.

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## BACTERIA OF THE VAGINA

There are two distinct portions of the vagina to consider bacteriologically, the vulva from the introitus to the ring of the hymen, and the true vagina from the ring of the hymen to the orifice of the uterus.

The *vulva* of the newborn is germ free. The observations of Neujean[1] indicate that bacteria appear on the external surfaces in seven to eight hours and are present after twelve hours in the deeper layers of the vagina. These organisms come from the vagina of the mother, from the bath water, and from the external world. The bacteria of the labia majora and minora are related to the bacterial flora of the immediate neighborhood. In the vestibulum a modified bacterial life exists, formed under the influence of the vaginal secretions and the vestibular glands. The bacteria from the outer world meet the specific vaginal secretions and the bacteria found in them, and in the resulting antagonism the latter gain the upper hand.

The secretion is probably the important factor in determining the flora of the vagina. This consists of the secretion of the glands in the vagina and in the cervix of the uterus, flat epithelial cells and leukocytes, except in the newborn. Bengelsdorf[2] and Neujean believe that the vaginal secretion of the newborn is always acid except when it is mixed with the amniotic fluid,

when it may be alkaline. Even then an acid reaction appears in twenty-four hours. The acidity increases rapidly, due possibly to the growth of Doederlein's acidophilic "vaginal bacillus." In 70 examinations of adult women Menge found an acid reaction forty-four times, an alkaline reaction nineteen times and an amphoteric reaction seven times. In gravid women the reaction is usually acid (Krönig and Walthard), but it may be neutral or alkaline. The acidity of the vaginal secretion of the newborn is attributed to $CO_2$ by Doederlein, that of adults to lactic acid, which may be as high as 0.4 per cent.

The vaginal secretion of the newborn and of healthy adults is strongly bactericidal. Thus Menge and Krönig[3] found that after the introduction of an emulsion of *Staphylococcus aureus* into the vagina of the newborn no organisms were present after fifty hours. Doederlein introduced the same organisms into the vagina of a virgin and found that they had disappeared in forty-eight hours. Menge put *Bacillus pyocyaneus* in the vagina of an adult and found that it was destroyed in twenty-one hours, staphylococci dying in twenty-six hours, streptococci in twenty-one to twenty-two hours. Similar experiments upon gravid women by Krönig gave practically the same result. Thus Krönig introduced *Bacillus pyocyaneus* twenty-six times, the staphylococcus sixteen times, and the streptococcus three times. All these organisms disappeared in seven to forty-eight hours. The streptococcus disappeared more rapidly than the others.

The bactericidal action of the vaginal secretions is subject to considerable variation. It may be diminished during menses and immediately after delivery, and is apparently strongest in gravid women. Both the acid and the alkaline secretions are bactericidal, so that the acidity, which is frequently high enough to kill streptococci (over 0.1 per cent.), is not the only factor. Phagocytosis may also take part in the destruction of organisms. The reactions are not entirely clear, but apparently the only organisms that can survive a long time in the vagina are those which withstand the bactericidal action of the secretions.

Examined microscopically the vaginal secretions reveal many bacteria, chiefly cocci, small rods, and delicate comma-shaped vibrios. Many of these fail to grow on artificial media, but occasionally small bacilli and white staphylococci can be cultivated. None of these organisms are pathogenic. Occasionally Doederlein's vaginal bacillus can be grown on acid agar, and a non-pathogenic streptococcus described by Krönig can be obtained by anaërobic methods.

*Pathogens.*—The presence of pathogenic bacteria in the normal vagina, especially in gravid women, has been investigated with some care, but the results are by no means conclusive. Thus Menge found in 70 non-gravid women that the specimens were sterile in 62 cases. In the other cases organisms like pigmented bacteria, non-pathogenic streptococci, and white staphylococci could be cultivated on alkaline agar. Probably all these organisms were introduced during the examination and the taking of the specimens. On acid agar Menge got Doederlein's vaginal bacillus, and by anaërobic methods Krönig's anaërobic streptococcus. Similar results have been obtained on gravid women by Krönig and by Williams,[4] who were unable to find streptococci in normal cases. It is thus evident that under normal circumstances pathogenic bacteria introduced into the vagina are rapidly destroyed, *i. e.*, the vagina normally cleanses itself. This self-purification is due in great measure to the powerful bactericidal action of the vaginal secretions, although phagocytosis of the bacteria by leukocytes may also be a factor. How far altered vaginal secretions in abnormal individuals

permit a development of pathogenic bacteria in the vagina is not clear, nor is it entirely certain that in women who do not observe proper rules of cleanliness, women who work in the fields, etc., the vaginal secretions are able to destroy the large numbers of bacteria which must be introduced into the vagina from time to time.

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## BACTERIA OF THE INTESTINAL TRACT

At the time of birth the intestinal canal of the normal child is free from bacteria and no organisms can be demonstrated in the meconium, either culturally or microscopically (Escherich,[1] Breslau[2]). Immediately after birth bacteria are introduced into the alimentary canal, probably by swallowing air which contains bacteria or by swallowing the secretions of the mouth, which are contaminated with bacteria soon after the child is delivered. The number and variety of bacteria that may be introduced depend upon the number and variety that are present in the child's environment, and the meconium soon after birth may reveal many different types of micro-organisms in small number. With the beginning of nourishment certain kinds of bacteria are introduced in excess and a well-defined intestinal flora begins to appear. This is subsequently influenced by the introduction of many different types of bacteria with food and drink as the child begins to take artificial nourishment, and gradually the characteristic bacterial flora of the adult is established. By the time the child is five years of age more or less constant conditions of feeding have been inaugurated, and from this time on the bacteria which may be found in the dejecta tend to become uniform in character.

**Bacteria in the Dejecta.**—Microscopic examination of the dejecta of the nursling reveals a mixed bacterial flora in which large and small Gram-positive bacteria are found in association with small Gram-negative bacteria, Gram-positive cocci, and occasionally yeast cells and bacterial spores. Many of these organisms can be brought to cultivation with ease, especially the Gram-negative bacteria, which are chiefly *Bacillus coli* and *Bacillus lactis aërogenes*, and the Gram-positive cocci, which are chiefly species of environmental origin or the organisms known as *Micrococcus ovalis* or the "enterococcus." The Gram-positive bacteria are more difficult to cultivate, often requiring special media and anaërobic methods. Many prove to be *Bacillus acidophilus* of Moro or *Bacillus bifidus* of Tissier. The large Gram-positive bacteria are principally sporulating organisms common to the environment like *Bacillus subtilis* or *Bacillus mesentericus*, but occasionally large Gram-positive anaërobes like *Bacillus Welchii* may be isolated. The spores present represent probably both aërobic and anaërobic species. According to Escherich putrefactive bacteria are uncommon in the stools of nurslings, possibly because they are inhibited by such species as *Bacillus bifidus* and *Bacillus acidophilus*.

In the child four to five years old fed on a *mixed diet* the bacteria of the dejecta are much more varied than they are in the nursling. A large number of species have been isolated from time to time and there is some confusion in the terminology. Thus in the five-year-old child Tissier[3] reports a constant flora in the dejecta, consisting of:

*Bacillus bifidus.*
*Bacillus acidophilus.*
*Bacillus exilis.*
*Bacillus III* of *Rodella.*
*Bacillus coli.*
The *enterococcus,*
and a variable flora which may reveal such organisms as:
*Bacillus perfringens* (*Bacillus Welchii*).
*Bacterium ventriosum.*
*Bacterium funduliforme.*
*Coccobacterium preacutum.*
*Coccobacterium oviforme.*
*Diplococcus orbiculus.*
*Staphylococcus parvulus.*
Yeast cells.

Observations made by the author[4] on the dejecta of children fed on a mixed diet reveal a surprisingly large number of organisms, but no such great regularity as is suggested by the work of Tissier. Moreover, much seems to depend on the character of the food and its richness in bacteria. In general we may distinguish microscopically both large and small Gram-positive bacteria like those seen in the nursling, the large Gram-positive bacteria being in excess, small Gram-negative bacteria in far greater numbers than the small Gram-positive organisms, Gram-positive and Gram-negative cocci, yeast cells, spores of great diversity in size and shape, occasionally branching organisms, and spiral forms. Many of these organisms can be cultivated without difficulty. Thus the Gram-negative bacteria are chiefly *Bacillus coli* and *Bacillus lactis aërogenes*, but the rarer intestinal bacteria like *Bacillus alcaligenes* are frequently encountered. The small Gram-positive bacteria are usually *Bacillus acidophilus* and *Bacillus bifidus.* Of the large Gram-positive bacteria the majority are environmental species like *Bacillus subtilis* and *Bacillus mesentericus.* Gram-positive anaërobes like *Bacillus Welchii* are nearly always present, usually in spore form. The spores apparently represent both aërobes and anaërobes. Both Gram-positive and Gram-negative cocci may be cultivated, practically always non-pathogenic species and probably derived from the environment. Rarely *Micrococcus ovalis*, the "enterococcus," can be obtained.

In general, the bacteria of the child fed on a mixed diet differ from those of the nursling in their larger number and variety and the relative predominance of Gram-negative organisms.

Bacteria are present in great variety and profusion in the dejecta of adults, and many attempts have been made to cultivate them and to offer some satisfactory scheme for their classification. Smears from the discharges reveal both Gram-positive and Gram-negative bacteria of various sizes, Gram-positive and Gram-negative cocci, spores of nearly every type, yeasts, molds, branching forms of streptothrix (actinomyces), spiral organisms, and occasional spirochetes. As first noted by Escherich, however, cultures on ordinary media yield chiefly *Bacillus coli* and *Bacterium lactis aërogenes.* Many of the organisms in the dejecta are dead, others are anaërobes which do not grow by ordinary methods, still others require special media for artificial cultivation. Possibly the great excess of certain species like *Bacillus coli* prevents the development of the rarer organisms on plates. With the introduction of many varieties of artificial media and the employment of special methods, the number of fecal bacteria which can be brought to cultivation has become very large (McNeal).

The following list gives the principal organisms which can be obtained from the dejecta by ordinary aërobic and anaërobic methods or by the use of special media:

1. Gram-negative bacteria, chiefly *Bacillus coli*, *Bacterium lactis aërogenes*, *Bacillus alcaligenes*, *Proteus vulgaris*, and rarely organisms belonging to the intermediate or paratyphoid group or liquifiers like *Bacillus Bookeri* of Sternberg.
2. Small Gram-positive bacteria like *Bacillus acidophilus*, or rarely *Bacillus bifidus*.
3. Anaërobic spore-bearing species like *Bacillus Welchii*, *Bacillus sporogenes*, *Bacillus putrificus*, etc.
4. Aërobic spore-bearing species like *Bacillus subtilis*, *Bacillus mesentericus vulgatus* and *fuscus*, *Bacillus megatherium*, etc.
5. Spore-bearing thermophils.
6. Vibrios and spirilla.
7. Spirochetes.
8. Gram-positive and Gram-negative micrococci, chiefly of environmental origin.
9. Gram-positive and Gram-negative streptococci.
10. Sarcinæ, chiefly Gram-positive and probably of environmental origin.

The number of bacteria given off in the dejecta is very large. Thus Gelbert and Dominicki estimate that 12 to 15 milliards are discharged daily. Sucksdorf[5] figures the number at 55 milliards, Klein[6] at 8800 milliards. According to Strassberger[7] man gives off daily 4.5 to 5.3 gr. of dried bacterial substance. That is, one-third to one-quarter of the fresh fecal material consists of the bodies of living or dead bacteria (McNeal[8]).

**Distribution of Bacteria in the Intestines.**—Before the establishment of the gastro-intestinal flora characteristic of adults there is no regularity in the distribution of bacteria in the alimentary canal. The organisms which are introduced with the food may develop in the stomach, pass rapidly through this organ, and appear in various parts of the gut. After the adult gastro-intestinal flora is once established more or less constant conditions arise and in general different individuals show only few or minor differences. The various articles of diet, both liquid and solid, are heavily infested with bacteria, only partially destroyed by ordinary cooking. Even the water constantly used by all individuals contains bacteria in considerable quantity. In addition the secretions of the mouth and nasopharynx are constantly mixed with the food as it is chewed and swallowed and are likewise swallowed independently. The contact of this germ-laden material with the lining of the esophagus is only temporary and the character of the stratified epithelium of that organ precludes any special development of organisms there. The microbic life in the intestines may be said therefore to begin in the stomach.

*Bacteria in the Stomach.*—Smears from the gastric contents removed during full digestion reveal bacteria of great number and variety. Large and small Gram-positive organisms predominate, mixed with a few Gram-negative bacteria, yeast cells, spores, vibrios, and spirochetes, with occasionally long-branching and non-branching threads. Many of these organisms represent the bacteria of the mouth, among which we may place the vibrios and spirochetes, the leptothrix threads, the small Gram-positive cocci, and some of the larger forms like *Bacillus buccalis maximus*. Other organisms like the large Gram-positive bacteria represent the aërobic spore-bearing species, *Bacillus subtilis*, *Bacillus mesentericus*, and *Bacillus megatherium*, with which our food is always contaminated under natural con-

ditions. Other Gram-positive bacteria are apparently acidophilic organisms derived probably from the mouth. The Gram-positive cocci are chiefly pigmented micrococci and sarcinæ introduced into the stomach with water and food, although some are evidently mouth pneumococci and streptococci. The Gram-negative bacteria are somewhat more difficult to place. Some are pigmented water organisms like *Bacillus fluorescens liquefaciens*, and *non-liquefaciens*, and occasionally they prove to be strains of *Proteus vulgaris*. In general, however, the Gram-negative bacteria are true intestinal species, especially *Bacillus lactis aërogenes* which is frequently implanted on the gastric mucosa. The spore material represents the spores of both aërobes and such anaërobes as *Bacillus Welchii*, *Bacillus sporogenes*, etc.

After gastric digestion is completed but few bacteria are left in the stomach, and those present represent merely a moiety of the forms there originally. This diminution in bacteria is to be traced to two factors, the high acidity which is sufficient to destroy bacteria of low resistance and the rapid elimination of bacteria with the food as it passes down into the duodenum.

*Bacteria in the Small Intestine.*—As the gastric contents pass down into the small intestine they carry with them the bacteria which have survived the action of the gastric juice. Their stay in the duodenum and upper part of the small intestine is of short duration and in general the contents of the small bowel reveal but few micro-organisms. Thus it has been shown by Cushing and Livingood[9] that the contents of the duodenum, jejunum, and ileum are practically sterile. In many instances, however, small Gram-negative bacteria, either *Bacillus coli* or *Bacterium lactis aërogenes*, are found implanted on the intestinal mucosa here.

*Bacteria in the Large Intestine.*—After preliminary digestion in the stomach and small intestine the food remnants accumulate in the cecum and colon in great quantity and the bacterial flora of this region is rich and varied. Smears from the contents of the gut and from the mucous membrane reveal a mixture of Gram-positive and Gram-negative bacteria, with the latter organisms in great excess. Occasionally Gram-positive and Gram-negative cocci, yeast cells, and spores may also be seen, and rarely spirochetes and branching organisms. Cultures on ordinary media yield almost universally *Bacillus coli*, frequently mixed with *Bacillus lactis aërogenes*. Occasionally some of the uncommon intestinal organisms like *Bacillus alcaligenes* or Morgan's bacillus may also be isolated. Sometimes the plates show a few isolated colonies of aërobic spore-bearing bacteria of the "subtilis" group, pigmented cocci or sarcinæ, and branching streptothrix threads. To bring to cultivation some of the other organisms seen in smears it is necessary to resort to special methods. By preliminary cultivation of the material in acid broth, by means of which the ordinary intestinal bacteria are destroyed, aciduric or acidophilic organisms can frequently be obtained like *Bacillus acidophilus* or some of the *Lactobacilli* such as *Bacillus bulgaricus*. Such species represent apparently some of the Gram-positive bacteria found in smears. Occasionally obligate anaërobes can be isolated by special technic, Veillon's agar, etc.

The character of the spores may be determined by heating material to 80° to 85° C. for half an hour and making aërobic and anaërobic plates. The aërobes obtained are usually members of the "subtilis" group and frequently conform in morphology to some of the Gram-positive bacteria found in films. The anaërobes are chiefly *Bacillus Welchii*, *Bacillus putrificus*, etc. It is impossible to determine by our present methods how much of this spore material represents spores originally present in the food which have not been

injured by the gastric juice and have passed through the stomach and small intestine, or aërobic and anaërobic species which have multiplied in the alkaline contents of the large bowel and produced spores there. While those findings may be said to represent the usual conditions in the large bowel certain organisms may be present in excess at times, even in normal individuals. Thus the ordinary intestinal bacteria are sometimes completely overgrown by *Proteus vulgaris* or *Bacillus pyocyaneus* and rarely the contents of the gut may reveal a great preponderance of Gram-positive cocci like non-pathogenic streptococci.

**Function of the Intestinal Bacteria.**—In the earlier days of bacteriology, when knowledge of the properties of bacteria was still in its infancy, the character of the intestinal flora was regarded as indifferent to the well-being of the individual and his digestive processes. This view was supported by the experiments of Nuttall and Thierfelder,[10] who in 1895 removed guinea-pigs from the mother by cesarean section and succeeded in keeping them alive for thirteen days on sterilized milk and germ-free cakes. While normal control animals with bacteria in the intestines gained 20.6 per cent. in weight by the end of the sixth day, the animals with germ-free intestines had gained only 11.75 per cent. On the tenth day the normal animals had gained 61.6 per cent.; the germ-free, 16.76 per cent.; while on the thirteenth day the normal individuals had increased 76.5 per cent.; the germ-free, 17.5 per cent. It was further observed that the animals removed by cesarean section and fed exclusively on sterilized cow's milk showed no gain on the sixth day, while normal animals showed a gain of 20.6 per cent. As a result of these experiments Nuttall and Thierfelder concluded that the presence of bacteria in the intestine is not necessary for the maintenance of life as long as animal food is given.

Subsequently Schottelius[11] conducted similar experiments with chicks, and found that while normal controls showed an increase in weight of 250 per cent. by the twelfth day, the chicks with germ-free intestines had an increase of only 24 per cent. as a maximum, and after the twelfth day lost weight rapidly and died. The longest duration of life in these animals with germ-free intestines was only thirty days. Furthermore, Schottelius found that when germ-free animals which had not gained weight were given food infected with *Bacillus coli* from cow's milk or from the intestinal tract of hens, they took on weight rapidly and caught up with normal controls. At the same time other animals given food infected with air organisms continued to lose weight and soon died. On the basis of these observations Schottelius concluded that chicks could not maintain life with germ-free intestines and that the same principle held true for all warm-blooded animals and for man. More recently Madame Metchnikoff[12] has obtained results like those of Schottelius with frog's larva and Moro[13] with turtles (Knoblauchkröte), and both observers came to the conclusion that intestinal bacteria were absolutely necessary for the life of cold-blooded animals.

Certain animals have no bacteria in the intestines, however, or such a small number that they can hardly have any function. Thus it has been shown by Lewin that intestinal bacteria are lacking in polar animals and yet normal digestion takes place. Metchnikoff[14] also has shown that cold-blooded animals like galeria tina and scorpions have no intestinal bacteria, but these observations have not been confirmed.

While the presence of bacteria in the intestines may not be absolutely essential for the maintenance of life, as Nuttall and Thierfelder have demonstrated, it must be remembered that bacteria of great number and variety are always found in the alimentary canal and that the abundant moisture

and equable body temperature maintained there are highly favorable influences for the multiplication of species which have very active and important properties. In view of this fact various observers have attributed certain reactions in the intestine to the action of micro-organisms. Thus Rodella[15] believes that in artificially fed children the intestinal bacteria play an important part in the peptonization of the casein of the cow's milk. Breton[16] thinks that *Bacillus coli* and *Bacillus lactis aërogenes* may take part in the tryptic digestion of albumin in early life before the digestive juices are fully formed. Metchnikoff suggested that in man the digestion of cellulose was accomplished by the action of bacteria, especially in early life, just as in certain animals where the food contains large quantities of cellulose the cellulose-splitting bacteria are present in abundance and have an important function.

While it is by no means clear that the bacteria in the intestines play a rôle in the earlier changes in the proteins, fats, and carbohydrates, as a result of which they become available for assimilation, the late changes in food materials, especially the putrefaction which takes place in the alkaline contents of the lower bowel, are largely to be attributed to the action of bacteria. Here many species are present and able to decompose proteins and produce from them such substances as indol, skatol, and phenol. Some of these species are ordinary aërobes like *Bacillus coli*, while others are putrefactive anaërobes, which are occasionally found in the large bowel. The constant production of skatol and indol in the lower gut and the lack of any digestive juices or ferments which can give rise to these substances indicate that the final degradation of protein is entirely to be attributed to bacterial action.

**Antagonistic Action in the Intestinal Bacteria.**—The building up of the normal intestinal flora of the adult depends upon various factors by no means clearly understood. Just why certain species like *Bacillus coli* and *Bacillus lactis aërogenes* come to be the predominating organisms in the alimentary canal of practically every variety of animal has never been entirely explained. It is evident, however, that conditions are proper for their survival, and certain observers, like Escherich, Jehle, and Pincherle,[17] believe that the body of each individual is suitable for a special *Bacillus coli*, which may not find a favorable nidus for development in other individuals. In favor of this view is the observation that different races of *Bacillus coli* differ serologically, but it is by no means certain that this serological difference can be correlated with residence of the individual strains of *Bacillus coli* in different persons.

Probably the most important factor in the establishment of the normal intestinal flora of the adult is the *antagonism* which certain species exert upon other species. Thus it was early pointed out by Bienstock that the great multiplication of *Bacillus coli* and *Bacillus lactis aërogenes* can prevent the development of the putrefactive bacteria of the intestine. Metchnikoff, Kolle, and Issaeff have found that intestinal bacteria play an important part in the survival or destruction of pathogenic bacteria. Thus young suckling rabbits, which have very few germs in the alimentary canal, can easily be infected by mouth with cholera vibrios, while young guinea-pigs, which take independent nourishment early and have a rich bacterial flora, can stand the same infection without injury. Somewhat similar observations have been made by Smith and Orcutt[18] in the disease known as scours in calves, in relation to *Bacillus coli*.

The effect which one species of bacteria in the intestines has upon the other species there may be due, however, not to a true antagonism, but to the action of certain products which the bacteria produce in the surrounding

medium. Thus Combe[19] found that the acids formed by the lactic acid bacilli of the intestine can prevent excessive intestinal putrefaction. A similar function was ascribed to *Bacillus bifidus* by Macfadyen, Nencki and Sieber,[20] and by Tissier and Martelly.[21] By the introduction of substances which favored the development of this latter species an excessive putrefaction was likewise prevented.

**Artificial Changes in the Intestinal Flora.**—The observation that the presence of different types of bacteria in the intestines of children depends to a large extent upon the kinds of food which are administered, together with the somewhat indefinite clinical experience that excessive intestinal putrefaction has a deleterious influence upon the health, has led to many different attempts to change the flora of the intestines by artificial measures designed to inhibit this putrefaction. It is by no means certain at present that this so-called excessive putrefaction is definitely injurious, and it is equally uncertain whether the therapeutic measures, by means of which the putrefaction may apparently be inhibited, have any definite effect upon the health.

Apparently Escherich was the first to suggest that acid-forming bacteria like *Bacillus lactis aërogenes* can be administered to children to check intestinal putrefaction, and a little later Quincke[22] employed cultures of yeasts, *Oïdium lactis*, for the same purpose. The subject was investigated more thoroughly by Metchnikoff, with whose work the so-called "lactic acid bacillus therapy" really started. In place of such an organism as *Bacillus lactis aërogenes* which produces but small quantities of acid from lactose, Metchnikoff[23] suggested the use of such a species as *Bacillus bulgaricus*, which ferments lactose with a larger amount of lactic acid (25 per cent.) and is employed in Bulgaria for the manufacture of buttermilk. Subsequently a number of other lactic acid bacilli have been employed for the same purpose and somewhat extravagant claims have been advanced as to their therapeutic value. As a result of numerous investigations it is now reasonably established that the constant administration of these lactic acid organisms has a definite effect upon the flora of the intestines, as a result of which Gram-positive organisms like those administered come to predominate in the dejecta. Whether there is a definite replacement of the ordinary intestinal bacteria by these Gram-positive organisms and an implanation of the latter upon the intestinal mucosa is not so certain. Thus during the period when the lactic acid organisms are constantly administered the stools may show an excess of Gram-positive bacteria. In many persons cessation of the administration of the lactic acid organisms is rapidly followed by a return of the stools to their normal character and the Gram-negative bacteria predominate. It has recently been shown, however, by Rettger[24] among others that in animals (rats) long-continued feeding with bacteria of the *Bacillus acidophilus* and *Bacillus bulgaricus* types apparently produces a permanent Gram-positive flora in the dejecta.

Somewhat similar changes in the fecal flora of man may be accomplished by feeding different articles of diet. Torrey[25] has shown that a diet rich in carbohydrates leads to an overgrowth of aciduric Gram-positive organisms and a suppression of proteolytic bacteria, while a rich protein diet leads to a suppression of the aciduric and an overgrowth of the proteolytic.

On the basis of these and similar observations it has been suggested that the changes in fecal flora which follow the administration of various types of sour milk containing lactic acid bacilli are, in reality, due to the lactose and lactic acid contained in the preparations and not to viable organisms.

**Pathogenic Organisms in the Intestines.**—Many of the ordinary intes-

tinal bacteria like *Bacillus coli* and *Bacterium lactis aërogenes* are occasionally virulent to small animals, but in general disease-producing bacteria do not occur in the contents of the normal intestinal tract. Rarely pyogenic cocci may be obtained from the stomach, but organisms of this character are usually destroyed by the gastric juice. In cases of typhoid fever, dysentery, and Asiatic cholera the etiological agents of these diseases are present in various parts of the gut and in the dejecta, and the same holds true for carriers in these diseases. In paratyphoid fever paratyphoid bacilli may be present in the discharges. Occasionally virulent streptococci are found, but in general the streptococci found under normal conditions are non-pathogenic. Especial interest attaches to the anaërobes that may be present in spore or vegetative form in the intestines. Thus *Bacillus Welchii* is practically always there, but no evidence has been brought to show that it has any pathological significance. Of special importance in this connection is the recent work of TenBroeck and Bauer,[26] who have shown that in certain parts of the Orient (Pekin, China) the tetanus bacillus may be cultivated from the dejecta of a large number of persons who have furthermore appreciable amounts of tetanus antitoxin in their blood.


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## Chapter II

# ENVIRONMENTAL BACTERIA

### BACTERIA IN THE AIR

In the early days of bacteriology much stress was placed upon the presence of bacteria in the atmosphere and it was generally believed that the etiological agents of infection were constantly distributed by air currents. This conception of the importance of air organisms was a natural consequence of Pasteur's work on fermentation, Lister's observations on wound infections, and Tyndall's experiments on the bacterial content of the air of different localities. Modern investigation has revealed that under ordinary circumstances the bacteria of the air are harmless saprophytes, pathogenic organisms being present, however, under exceptional conditions. The types of organisms may easily be demonstrated by exposing agar or gelatin plates for different periods of time to the air of various localities and incubating them at 37° or 22° C. The colonies which grow are usually pigmented bacteria like *Sarcina lutea*, spore-bearing species like *Bacillus subtilis* and *Bacillus mesentericus*, molds and yeasts and branching organisms which are now usually classed with the actinomyces. A rough indication of the number of bacteria in the air can be obtained by such plates, since the number of colonies represents the number of bacteria dropping on the plates in the period of exposure. In general, more colonies are found when the plates are incubated at 22° C. than at 37° C. since the air bacteria grow better at room than at body temperature. It is further to be noted that many of the colonies on ordinary air plates are made up of two or three kinds of organisms, suggesting that the bacteria are not present in the air as single individuals, but as clumps which may contain several different types. A more accurate method of determining the number of bacteria in the air was devised by Miquel.[1] A known quantity of air was drawn through sterile water; the number of bacteria in the water was then determined by making plates in the ordinary way and counting the colonies. Or the air may be drawn through fluid nutrient gelatin as recommended by Hueppe. Possibly the most accurate method of determining the number of air organisms is that of Hesse,[2] who employed a glass tube of known dimensions, 70 cm. long and 3.5 cm. in diameter, the inside of which was covered with a thin layer of gelatin. Air was drawn into this tube by suction, usually in $\frac{1}{2}$-liter quantities. The bacteria in the air fall on the gelatin covering the sides of the tube and the number may easily be determined after incubation for two or three days.

A known volume of air may also be sucked through sand, which is then washed in water or salt solution and the bacteria counted by the usual method (Zinsser).

The number of bacteria in the atmosphere varies greatly from time to time and from place to place. In general, the air of crowded cities is more heavily charged with bacteria than the air of rural districts, but the differences are not striking. The number is also dependent upon the air currents and upon the moisture of the surfaces upon which the air impinges. It has been shown by Flügge[3] that bacteria are not lifted by air currents from moist surfaces. It is probable that the majority of the micro-organisms in the atmosphere cling to dust particles. After rain storms, when the air is washed free from dust, the bacterial content is very low.

The distribution of air bacteria has been studied especially in balloon ascents. Below altitudes of 1500 feet organisms are always present and small numbers may be found even as high up as a mile or more above the earth's surface.

The bacteriology of the atmosphere is of practical importance chiefly in regard to *aërial infection.* It is generally believed that some infectious diseases can pass from person to person through the atmosphere. This is particularly true in regard to certain of the exanthemata like smallpox and measles. The evidence that such aërial transmission actually takes place is not very satisfactory, and since we have no knowledge of the etiological agents of these diseases the mechanism of transmission is still more doubtful. With known infectious agents two important lines of investigation have given us some knowledge as to their passage through the atmosphere. It has been demonstrated by Flügge[4] and his associates that moist particles containing bacteria are expelled from the body in coughing and sneezing, and in ordinary speaking, although to a less extent. Kirstein,[5] Hutchison,[6] and others state that some of these particles are extraordinarily small and light and can be transported great distances by currents of air of low velocity, 0.1 mm. per second. Under such conditions the bacteria would not be injured. Subsequently M. Neisser[7] has attempted to determine the extent to which pathogenic bacteria dried down to the form of dust are carried in a living condition by air currents having a velocity of 4 meters per second (about the velocity of air currents in well-ventilated rooms) up to a height of 80 cm. or more. Only tubercle bacilli, staphylococci, anthrax, and tetanus spores were thus transported in a living condition. With air currents of greater velocity typhoid bacilli and streptococci were also moved alive. Cholera vibrios, pneumococci, plague bacilli, diphtheria bacilli, and influenza bacilli all died out under these conditions. An aërial transmission of these organisms is thus ruled out under ordinary conditions such as prevail in the atmosphere of closed rooms. With currents of air of greater velocity produced by winds, the sweeping of floors, the dusting of furniture, etc., the bacteria in the air are naturally moved to greater distances. In all cases they settle rapidly to the floor.

As to the length of time during which pathogenic bacteria survive in the atmosphere we are still ignorant. Bacteria are rapidly destroyed by drying and sunlight, and moist particles conveyed by the atmosphere would naturally be more infectious than dried particles of dust. Indeed, it has been pointed out by Koblisch that the infectivity of the tubercle bacillus is greater under moist conditions than when dried down. It is now taught that pathogenic organisms discharged with the secretions and excretions of man and animals actually get into the atmosphere only under exceptional conditions and survive there for only limited periods of time. The uniformity of the results of bacteriological examination of the atmosphere from widely separated localities and the usual absence of pathogenic bacteria testify to the correctness of this view. Thus Forbes[8] has recently shown that there are no more bacteria in the air of the underground electric tunnels of London than in the atmosphere outside.

1. Miquel: Ann. d. l'observ. de Montsouris, 1879, 1885, 1886.
2. Hesse, W.: Mitthe. s. d. K. Gsndhtsamte, 1884, ii, 182–207.
3. Flügge, C.: Ztschr. f. Hyg., 1897, xxv, 179–224.
4. ———: Ztschr. f. Hyg., 1899, xxx, 107–124.
5. Kirstein, F.: Ztschr. f. Hyg., 1900, xxxv, 123–164.
6. Hutchison, R. F.: Ztschr. f. Hyg., 1901, xxxvi, 223–253.
7. Neisser, M.: Ztschr. f. Hyg., 1898, xxvii, 175–200.
8. Forbes, J. G.: J. Hyg., 1924, xxii, 123–155.

## BACTERIA OF THE SOIL

The soil may be regarded as the home of the bacteria, the area from which they come, and the area to which they eventually return. Here bacteria are deposited in enormous numbers from the secretions of men and animals, from cadavers, and from putrefying material of all sorts. From the soil the bacteria pass into water, are lifted by currents of air into the atmosphere, reach the surfaces of the body of man and animals, get on all growing plants, contaminate the food we eat and the clothing we wear. There is thus a constant movement of bacteria which leave and return to the soil in a never-ending stream.

The bacterial content of the soil varies markedly in different areas and in different parts of the world. It depends somewhat on climatic conditions, the multiplication of the bacteria being favored by high temperature and by moisture, and hindered by cold and dryness. The superficial layers are richer in bacteria than the deeper. Fraenkel[1] has found that the bacteria are most numerous near the surface and gradually diminish, down to a depth of about two yards. Below this the soil may be practically sterile. The bacterial content also varies greatly with the type of life existing above it. In thickly populated areas bacteria are deposited in great numbers both by man and animals. In desert districts this source of contamination is lacking and the number of bacteria in the soil is correspondingly less. Sewage-polluted soil may contain 115,000,000 bacteria per gram; garden soil, 1,500,000 per gram, and uncultivated sand soil, only 100,000 per gram (Houston[2]).

Two main groups of bacteria may be distinguished in the soil. On the one hand, we have the true *soil bacteria* which live exclusively in the soil and play an important rôle in the complex biological processes which take place there. On the other hand, we have the *adventitious bacteria* that are occasionally deposited in the soil and find there conditions which permit their survival.

We may distinguish three main types of the true soil bacteria:

1. *Nitrifying bacteria.*

These bacteria change ammonia compounds to nitrates by a process of oxidation, the nitrates formed being available for plant life. The ammonia is changed first to nitrites and these are then changed to nitrates. The principal species active in these changes are the nitrifying bacteria of Winogradsky, *Nitrosomonas europea* and *javanensis Nitrobacter Winogradskyi*, and the *Nitrosococcus.*

2. *Denitrifying bacteria.*

These bacteria reduce the nitrates to nitrites and then to ammonia and even to free nitrogen. Gaseous oxids of nitrogen are also produced. The bacteria concerned in these changes are chiefly the spore-bearing anaërobes like *Bacillus sporogenes* and *Bacillus putrificus.* How far the sporulating aërobes and non-sporulating species like "proteus," which exist in the soil in great numbers, bring about these changes is not clear.

3. *Nitrogen-fixing bacteria.*

Several types of soil organisms can fix nitrogen and make it available for plant life. In some instances the nitrogen is fixed when the bacteria live in solutions containing carbohydrates and poor in combined nitrogen. The principal species active in this process is *Azotobacter chroococcus* of Beijerinck. In some instances this fixation of nitrogen takes place in the root nodules of leguminous plants, the principal species bringing about this change being *Bacillus radicicola* (*Rhizobium leguminosarum* Frank). Winogradsky has demonstrated that spore-bearing anaërobes like *Clostridium pasteurianum*

can also fix nitrogen outside the plant roots, their power to fix nitrogen being increased in the presence of sugar and diminished when nitrogenous substances are present. Other nitrogen-fixing bacteria have been described by Bailey.

Of the adventitious bacteria of the soil, as distinguished from true soil bacteria, a great number of species have been described from time to time. Possibly the most common are the aërobic spore-bearing bacteria like *Bacillus subtilis*, *Bacillus cereus*, *Bacillus mesentericus*, and *Bacillus megatherium*, frequently present in soil samples gathered from a variety of sources. Next to these come the spore-bearing anaërobes like *Bacillus Welchii*, *Bacillus sporogenes*, and *Bacillus tetani*, which may be encountered in abundance in richly manured soils. Finally, there are many pigmented bacteria, branching threads, molds, yeasts, and thermophils which may be isolated from time to time in the upper layers of the soil. Such organisms as these adventitious species are of chief interest to man in that they are the principal contaminating organisms in our foods, in milk, canned goods, etc.

The importance of the soil to the medical and public health bacteriologist attaches to the presence of well-known pathogenic bacteria there which may incite disease in man and animals. Of the pathogenic aërobes the most important is *Bacillus anthracis*, the cause of anthrax or splenic fever of cattle. This organism is widely distributed in soils where large numbers of diseased animals have been pastured, especially in certain areas of central Europe, like France and Germany, and in certain South American countries, like Argentina. In such areas anthrax is common in cattle, less frequent in sheep, and the soil is constantly receiving fresh accessions of this bacillus from the infected animals and especially from their cadavers. Of the pathogenic anaërobes the most important organism is the tetanus bacillus which exists in many different soils in various parts of the world. It is possible that this organism is also constantly returned to the soil in the dejecta of man and animals as has been shown by TenBroeck and Bauer[3] for China. Other pathogenic anaërobes of interest are *Bacillus Welchii*, *Bacillus chauvei*, *Bacillus sporogenes*, *Vibrion septique*, *Bacillus histolyticus*, *Bacillus tertius*, etc., active agents in wound infections in man and animals, where the surfaces of the wounds become contaminated by soil. Finally, *Bacillus botulinus*, the multiplication of which in foods is accompanied by the production of a deadly toxin, is present in the soil of many different parts of America and of Europe (Meyer), and from the soil easily passes into foods in the process of canning and preservation.

Of the non-spore-bearing bacteria our chief interest lies in the organisms derived from the dejecta in health and disease, particularly *Bacillus coli* and *Bacillus typhosus*. In areas where the soil is polluted with sewage and with the dejecta *Bacillus coli* is not uncommonly present. To what extent this species finds conditions for actual multiplication in the soil is not certain, since it may merely be deposited there and survive for a limited period. Firth and Horrocks[4] have found that the typhoid bacillus under special circumstances may remain alive in soil for some time, possibly several months. Such highly specialized parasitic bacteria of man as the pneumococcus, the meningococcus, etc., do not apparently survive except for the briefest periods. How far pathogenic acid-fast organisms remain alive in the soil after deposition there is not clear, since the soil normally contains representatives of this group which may have some virulence for animals.

In the cadavers of persons dead of infectious diseases and then buried pathogenic organisms like the typhoid bacillus and the cholera vibrio die within two to three weeks (Petri[5]). The tubercle bacillus usually dies within

three months. In unpublished observations by the late Wyatt Johnson in Montreal tubercle bacilli were demonstrated microscopically in the lungs of a man dead of pulmonary tuberculosis whose body had been buried for several months.

The number of bacteria in the soil may be determined by counting colonies on agar or gelatin plates in the usual way. Such plates bring to cultivation the species which grow on the usual media. The quantity of soil should be accurately weighed and the number of bacteria estimated per gram. For the cultivation of the true soil bacteria, *i. e.*, the forms which play a rôle in the biological changes in the soil, special synthetic media must be employed, since these species do not grow on ordinary media. Thus for the nitrifying bacteria silica jelly must be used, for the actinomyces such substances as Czapek's agar. In order to estimate the bacterial content of soil at different layers samples must be obtained in a special apparatus by the use of which contamination with the bacteria from other layers may be avoided. A drill devised by Fraenkel is convenient for this purpose. Since many of the bacteria in the soil are obligate anaërobes, both aërobic and anaërobic methods should be employed in its study.

In *dust* the bacteria present are chiefly the ordinary spore-bearing organisms of the soil and the pigmented bacteria of the atmosphere. In the dust of crowded rooms occasionally such organisms as the pneumococcus may be found. In rooms where people with open tuberculosis are living the tubercle bacillus may rarely be present, especially in dark corners not reached by the air and sunlight where some moisture survives. In rooms where large numbers of people are working together, as in crowded factories, intestinal bacteria are constantly present in the dust. Thus in unreported observations made by the author on large factories, where hundreds of women were employed at hand work, samples of material collected from the floors, from the tops of tables, and from various utensils used by the employees practically always contained *Bacillus coli* as the predominant species.

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2. Houston, A. C.: Rep. Med. Off. Local Gov. Bd., London, 1897.
3. TenBroeck, C., and Bauer, J. H.: J. Exper. M., 1923, xxxvii, 479–489.
4. Firth, R. H., and Horrocks, W. H.: Brit. M. J., 1902, ii, 936–942.
5. Petri: Arb. a. d. K. Gsndhtsamte, 1891, vii, 1–31.

## BACTERIA IN WATER

The presence of bacteria in water has been recognized from the beginnings of bacteriology, but Koch was probably the first to suggest that their number might be of value in determining the sanitary quality of drinking-water. We now know that all varieties, the surface water of streams, rivers, and lakes, superficial and deep wells, contain bacteria the number and kinds of which depend upon many factors. In water exposed to the atmosphere the bacteria of the air drop on the surface, while the organisms in the superficial layers of the soil are washed up and added to it. In well water they are derived chiefly from the soil. The water from superficial wells has a higher content than that of deep wells, since it receives organisms from a soil more richly permeated with bacterial life. Ground water, *i. e.*, deep well water, contains relatively few organisms, since the bacteria are filtered off by the soil through which the water passes. Indeed, deep ground water may be practically sterile. Water from artesian wells sunk in deep layers of rocks is usually free from micro-organisms, as is the water of the so-called artesian wells sunk deep in sand. Rain water contains but a small number of bacteria, only those species present in the air through which the rain falls. In salt

water special varieties are found which grow in the presence of salt, particularly the luminescent or "phosphorescent" bacteria.

**Types of Bacteria in Water.**—Many different kinds of bacteria have been isolated from water and may be considered normal to it. These organisms are derived chiefly from the atmosphere, from the soil, and from decaying vegetation. They are quite without significance and in general have no pathogenic action upon animals or man. These organisms may be grouped as follows:

1. Pigmented micrococci and sarcinæ, like
   *Micrococcus cremoides*,
   *Sarcina lutea*.
2. Pigmented and non-pigmented bacteria, like
   *Bacillus fluorescens liquefaciens*,
   *Bacillus fluorescens non-liquefaciens*,
   *Proteus vulgaris*, etc.
3. Spore-bearing aërobic bacteria derived primarily from the soil, such as
   *Bacillus subtilis*,
   *Bacillus cereus*,
   *Bacillus mesentericus*,
   *Bacillus megatherium*,
   *Bacillus mycoides*,
4. Spore-bearing anaërobes, like
   *Bacillus Welchii*,
   *Bacillus sporogenes*,
   *Bacillus tertius*,
5. Cholera-like vibrios.
6. Spore-bearing thermophils.
7. Branched forms grouped with the actinomyces.
8. Yeasts and molds.

**Number of Organisms in Water.**—For the determination of the number of bacteria in water colony counts may be made on agar and gelatin incubated at 37° or 22° C. In general, gelatin counts at 22° C. give a truer idea of the number of bacteria living in the water, since the species normally present grow better at low temperatures. Counts vary greatly with the type of water, the season of the year, the amount of organic material, and the sedimentation which has taken place.

In rain water but few bacteria are present, usually less than 20 per cubic centimeter (Miquel[1]), and these are the organisms of the atmosphere. In good spring water the count is usually low, less than 100 per cubic centimeter. In superficial wells the count is higher and may run up to 500. In surface waters where there is a good deal of decaying vegetation the count tends to be still higher, rarely as much as 2000 to 2500 per cubic centimeter. Finally, in streams polluted by the fecal discharges of man or animals large numbers of bacteria may occasionally be present, 5000 to 50,000 per cubic centimeter, and sometimes even more. After sedimentation the bacterial count comes down to a lower level.

**Pathogenic Organisms in Water.**—The important pathogenic organisms which may be present in water are the cholera vibrio and the typhoid bacillus, and repeated attempts have been made to isolate these species and to devise methods by means of which they can be obtained with regularity. How far the vibrio of Asiatic cholera survives in water is debatable. The early examinations revealing its presence were carried out at a period when identifications were by no means as exact as they are today, and it is generally believed that cholera-like vibrios normally present in water were mistaken

for the true comma bacillus. Definite isolations of the cholera vibrio from water have been reported by Dunbar and by Loeffler.[2]

Attempts to obtain the cholera vibrio from water should be carried out with special enrichment methods, media similar to those employed for the isolation of the organism from the dejecta of infected individuals being used.

The typhoid bacillus is undoubtedly present in water with great frequency since accurate epidemiological investigation indicates that water polluted with this organism may be one of the chief sources of typhoid fever. Actual isolations of the typhoid bacillus from water are, however, surprisingly few. Wilson[3] accepts only about half a dozen instances in which the organisms regarded as the typhoid bacillus have been identified by modern methods, including cultural and serological reactions. In some instances the water has been obtained almost immediately after its pollution by the discharges of known typhoid patients. In other cases the water of polluted streams has been filtered, the viscid material collecting on the filters planted on special media and a few colonies of the typhoid bacillus obtained. In general, attempts to isolate the typhoid bacillus from water are practically fruitless.

The anthrax bacillus is present occasionally in streams which receive the refuse from tanneries. It may be isolated by heating samples of water to 80° to 90° C. for a few minutes to destroy the vegetative bacteria and making agar or gelatin plates from the heated material. While the colonies of the anthrax bacillus are characteristic, transfers should always be made and suspicious organisms tested for pathogenicity upon animals. Some of the non-pathogenic spore-bearing species in water occasionally resemble *Bacillus anthracis*.

Brown, Petroff, and Heise have pointed out that the stream of water which receives the sewage of Saranac Lake, N. Y., occasionally contains the tubercle bacillus.

Many of the organisms pathogenic to man, such as the pneumococcus, meningococcus, influenza bacillus, etc., apparently do not survive in water and have not been isolated from it.

**Sanitary Quality of Water.**—The sanitary quality of water, that is, its fitness for drinking purposes and its freedom from pathogenic micro-organisms, is now determined by estimating the number of bacteria that may be found and upon the presence or absence of intestinal bacteria, as shown by the presence or absence of *Bacillus coli*. With the passage into water of the fecal discharges of man and animals the number of bacteria is directly increased. At the same time the organic material in the water is augmented and the organisms already present there are provided with the proper nutritive material for their multiplication. A safe standard for drinking-water is 50 to 100 bacteria per cubic centimeter. Higher counts are sometimes permissible when the water is demonstrably free from fecal pollution, while strict standards are sometimes necessary when a constant stream of fecal material is passing into a large body of water. A sudden increase in the number of bacteria in water is always a matter of suspicion and its cause should be investigated.

**Bacillus Coli in Water.**—To determine the presence of *Bacillus coli* in water, plates may be made with media which favor the development of this organism. The best material for this purpose is lactose-litmus agar, or Drigalski-Conradi medium, Endo-agar, and eosin methylene-blue agar. Plates are made in the regular way, usually with dilutions of the water, and the number of *Bacillus coli* colonies counted. These colonies should be transferred to standard media and the properties of the organisms determined. For the actual demonstration of the presence of *Bacillus coli* suspicious

organisms should be identified by morphology, tinctorial properties, and cultural reactions.

The presence of *Bacillus coli* in water may better be determined by inoculating lactose Smith fermentation tubes with known quantities of the water, 5 c.c., 3 c.c., 1 c.c., or 0.1 c.c. The production of gas in the tubes and an acid reaction indicate that fermenting organisms are present. This is called a positive *presumptive test.* The actual proof of the presence of *Bacillus coli* is obtained by making Drigalski-Conradi, Endo, or eosin methylene-blue plates from the tubes which give positive presumptive tests, transferring characteristic colonies and identifying them by the usual methods. The finding of micro-organisms which give the typical reactions of *Bacillus coli* is called the *confirmation* of the *presumptive* test.

The presence of *Bacillus coli* regularly in 1 c.c. quantities of water is usually an indication that the water is receiving fecal pollution. Its presence in smaller quantities may be regarded as evidence of serious contamination. Its occasional presence in larger quantities, 3 c.c. and 5 c.c., should be made the occasion for carefully scrutinizing the sources of the water supply.

In the examination of water by the Standard Methods of the American Public Health Association[4] counts are made from gelatin plates incubated for forty-eight hours at 20° C. in the dark or from agar plates incubated twenty-four hours at 37° C. in the dark. It is advised that plates showing 30 to 300 colonies be employed for the counts and that these counts be made from at least two plates. Counting should be done with a lens with a magnification of $2\frac{1}{2}$ diameters ($2\frac{1}{2}$ X) with a focal distance of $3\frac{1}{2}$ inches. For the determination of the presence of fecal organisms it is recommended that the *Bacillus coli* group include all non-spore-forming bacilli which ferment lactose with gas formation and grow aërobically on standard solid media. The formation of 10 per cent. or more gas in a standard lactose broth fermentation tube within twenty-four hours at 37° C. is regarded as *presumptive* evidence of the presence of numbers of the *Bacillus coli* group. The appearance of aërobic colonies which ferment lactose in Endo or eosin methylene-blue plates made from positive lactose broth fermentation tubes is regarded as *partially* confirming the presumptive test. The *complete* confirmation is the final demonstration of the presence of members of the *Bacillus coli* group. This is shown by the inoculation of lactose fermentation tubes by typical colonies from the Endo or eosin methylene-blue plates, which should again ferment the lactose with gas formation. It is further recommended that the standard tests for the *Bacillus coli* group be A the *presumptive*, B the *partially* confirmed, or C the *completed* tests.

For the differentiation of fecal from non-fecal members of the *Bacillus coli* group as above defined it is recommended that at least 10 cultures be used, preferably from plates made directly from the water, otherwise from plates made from positive fermentation tubes. *Bacillus coli* of fecal origin is defined as the group of organisms which do not liquefy gelatin, fail to ferment adonite and usually saccharose, give a positive methyl red reaction, do not give a Voges-Proskauer reaction, and usually produce indol. The *Bacillus aërogenes* of fecal origin is defined as methyl red negative, Voges-Proskauer positive, gelatin negative, adonite positive, indol usually negative, saccharose positive. Organisms which differ from the last group only in failing to ferment adonite are regarded as *Bacillus aërogenes* presumably not of fecal origin. *Bacillus cloacæ*, which is methyl red negative, Voges-Proskauer positive, liquefies gelatin, ferments adonite and saccharose, and does not usually produce indol, may or may not be of fecal origin.

Of special importance in connection with the bacteriological examination

of drinking-water are the reports of the United States Public Health Service in which the standards established for drinking-water on common carriers are described.

In the first report[5] the following maximum limits of permissible bacteriological impurity were outlined:

1. The total number of bacteria developing on standard agar plates, incubated twenty-four hours at 37° C., shall not exceed 100 per cubic centimeter, provided that the estimate shall be made from not less than two plates, showing such numbers and distribution of colonies as to indicate that the estimate is reliable and accurate.

2. Not more than one out of five 10 c.c. portions of any sample examined shall show the presence of organisms of the *Bacillus coli* group.

The presence of these organisms is to be determined by planting five 10 c.c. portions in fermentation tubes containing not less than 30 c.c. lactose peptone broth and incubating forty-eight hours at 37° C. From the tubes showing gas, more than 5 per cent. of the closed arm of the fermentation tube, plates are made after forty-eight hours' incubation upon lactose litmus agar or Endo's medium. From these plates characteristic colonies are fished and tested in general according to the specifications of the Standard Methods of Water Analysis (A. P. H. A., 1912).

In the last report[6] the so-called Treasury Standard provides that the water shall be obtained from a source free from pollution, from a source adequately protected by natural agencies from the effects of pollution, or adequately protected by artificial treatment. The water-supply system, reservoirs, pipes, etc., shall be free from sanitary defects. The bacteriological examination of the water shall be carried out with a *standard sample* of 5 fermentation tubes inoculated with *standard portions* of 10 c.c. of the water under examination. The *Bacillus coli* group is defined by the Standard Methods of Water Analysis of the American Public Health Association (1923), namely, "as including all non-spore-forming bacilli which ferment lactose with gas formation and grow aërobically on standard solid media."

The following regulations as to the bacteriological quality of the water have been adopted (April 10, 1925):

1. Of all the *standard* (10 c.c.) *portions* examined not more than 10 per cent. shall show the presence of organisms of the *Bacillus coli* group.

2. Occasionally three or more of the five equal (10 c.c.) *portions* constituting a single *standard sample* may show the presence of *Bacillus coli*. This shall not be allowable if it occurs in more than

(*a*) Five per cent. of the *standard samples* when twenty (20) or more samples have been examined;

(*b*) One *standard sample* when less than twenty (20) samples have been examined.

In addition to the proper bacteriological condition, the water should be clear, colorless, odorless, and pleasant to the taste, and should not contain an excessive amount of soluble mineral substances nor of any chemicals employed in treatment.

Eijkman[7] claims that the sanitary quality of water can be determined by the use of fermentation tubes containing an aqueous solution of 10 per cent. glucose, 10 per cent. peptone, and 5 per cent. NaCl, inoculated with small quantities of water and incubated at 46° C. This test rests on the idea that thermo-tolerant bacteria which can multiply and ferment sugar at this high temperature are characteristic of the intestinal tract of warm-blooded animals. A positive fermentation indicates the presence of fecal contamination. The Eijkman test, together with tests for indol, is now

employed in Germany to determine the sanitary condition of the drinking-water.

Various methods have been devised to destroy or remove the pathogenic organisms in water. It may be treated with such oxidizing agents as potassium permanganate which serves to destroy the bacteria in small quantities of water, although giving it a decided color. The water may be treated with hypochlorite of soda or with liquid chlorin. Or the organisms may be destroyed by ultra-violet rays. For removal of bacteria water may be filtered through slow sand filters or through rock filters by the rapid method.

**Ice.**—Artificial ice, especially when made from distilled water, usually contains but few bacteria. Natural ice, collected from the surface of contaminated water, has been regarded as a source of typhoid infection by a number of authors, and Prudden[8] has shown that the typhoid bacillus can survive some months when frozen into ice. Chapin[9] states when natural ice forms on the surface of polluted water, the majority of the bacteria are frozen out, while those not so destroyed tend to die off rapidly. The danger of infection from ice has apparently been much exaggerated.

## BACTERIA IN SEWAGE

Since sewage consists chiefly in the dejecta of man largely diluted with water, the bacterial count is uniformly high. It runs up to 30,000,000 to 50,000,000 bacteria per cubic centimeter in large cities. Lower counts are also common, 3,000,000 to 10,000,000 per cubic centimeter. In many instances the counts are higher in summer than in winter. The types of bacteria conform closely to the types of organisms found in the feces of man. *Bacillus coli* is the predominating organism. Occasionally species like *Bacillus cloacæ* of Jordan are also isolated. Resistant spore-bearing bacteria belonging to the subtilis group are also very common. Sewage is usually examined, especially in America, by the methods employed for water analysis, namely, total count and estimations of *Bacillus coli.* In England much stress has been laid upon the presence of sewage streptococci and such anaërobes as *Bacillus enteritidis sporogenes* of Klein.

Pathogenic organisms like the cholera vibrio and the typhoid bacillus do not apparently survive in sewage, since they have not been isolated from it.

1. Miquel, P.: Rev. d'hyg., 1886, xviii, 388–392.
2. Loeffler: Centralbl. f. Bakteriol. [etc.], 1893, xiii, 380–385.
3. Willson, H. S.: J. Hyg., 1905, v, 429–443.
4. Standard Methods for the Examination of Water and Sewage. 5. ed. rev. New York, 1893.
5. Pub. Health Rep., 1914, xxix, ii, 2059–2066.
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## BACTERIA IN MILK

The milk of various animals is used for food by man, chiefly cow's milk in America, goat's milk in parts of Europe and in the Orient, mare's milk in certain regions of the Far East, reindeer's milk in Iceland. The almost universal use of cow's milk, and the ease with which it may be contaminated by bacteria and give rise to disease in man, make the study of the microorganisms present in it under both normal and pathological conditions one of our most important public health undertakings. In the mammary gland of the cow the lacteal secretions are probably sterile. Milk as it comes from

the udder, however, is practically always contaminated by bacteria which come from the skin about the orifices of the ducts. By carefully catheterizing the ducts and withdrawing the milk under aseptic precautions samples of sterile milk may be obtained, but only in a small proportion of cases (Bergey).

Immediately after milk is withdrawn from the animal it receives bacteria from various sources, among which the most important are the following:

1. The skin of the udder which is always bathed by the intestinal discharges of the animal.
2. The air and dust of the stable.
3. The utensils in which the milk is received and stored.
4. The hands of the milker.

In consequence of this prolific source of micro-organisms milk from normal animals always contains bacteria, the number of which depends upon the care with which the milk is handled and the period of time which has elapsed since milking. While in all probability the types of bacteria in different localities may differ somewhat, the species actually found in samples of milk from widely separated localities are much the same. We may distinguish about ten different kinds of bacteria that may be found in almost any sample of cow's milk provided sufficiently large quantities of milk are examined and the search be sufficiently prolonged.

1. Large Gram-positive bacteria representing aërobic spore-bearing species like *Bacillus subtilis*, *Bacillus mesentericus*, *Bacillus cereus*, *Bacillus albolactis*, etc. These organisms come chiefly from the dust of the stable and from the intestinal discharges of the animal in which spore-bearing bacteria are especially abundant.

2. Gram-positive bacteria representing anaërobic species like *Bacillus sporogenes*, *Bacillus butyricus*, *Bacillus Welchii*, *Bacillus tertius*, etc. These anaërobes come also from the intestinal discharges of the cow and from the dust of the stable.

3. Smaller Gram-negative bacilli, principally *Bacillus coli* and *Bacterium lactis aërogenes*. These organisms are derived from the dejecta of the animal and occasionally from the hands of the milker.

4. Gram-positive streptococci, usually without pathogenic action on animals and able to produce large quantities of lactic acid from lactose. The milk streptococci are now grouped together as *Streptococcus lacticus*. These organisms probably come from the skin of the udder, but may be derived from the dejecta.

5. Pigmented Gram-positive and Gram-negative bacteria of great variety derived primarily from the air of the stable.

6. Gram-positive micrococci and sarcinas, usually pigmented, coming chiefly from the air.

7. Gram-positive, spore-bearing thermophils.

8. Spores of various sizes and shapes, representing both aërobic and anaërobic species.

9. Yeast cells.

10. Molds and branching organisms grouped with the actinomyces.

**The Number of Bacteria in Milk.**—The extent to which milk is contaminated by bacteria may be determined by making agar or gelatin plates of proper dilution and counting the colonies. It is important to select plates for counting which are not too crowded with colonies and which show a uniform distribution. Agar plates incubated at 37° C. give the most information in regard to the intestinal bacteria, while gelatin plates at 22° C. are better for the determination of the organisms from the air and dust. The

number found in different samples varies enormously. In milk obtained with the greatest care, the udders of the animals being washed, the utensils properly constructed with small tops and sterilized before use, the hands of the milker washed and disinfected, the count may be very low in fresh samples, less than 1000 per cubic centimeter or even down to 500 per cubic centimeter. In milk obtained without these precautions, but with ordinary attention to cleanliness, the count is higher, often 5000 to 10,000 per cubic centimeter. Finally, in milk gotten carelessly, little or no attention being paid to methods of insuring cleanliness, the count may run up to 50,000 to 100,000 per cubic centimeter. In old milk the bacteria are very numerous and occasionally 500,000 to 1,000,000 per cubic centimeter may be present.

A simple method of counting the bacteria in milk has been devised by Prescott and Breed.[1] Here small quantities of milk, preferably 0.01 c.c., are spread over microscopic slides measuring 1 sq. cm. These films are dried in the air, fixed with methyl alcohol, and treated with xylol to dissolve off the fatty constituents. They are then stained lightly with Jenner's stain or ordinary methylene-blue. The number of bacteria in a microscopic field of 1/50 sq. mm. or 0.0002 sq. cm. is counted and the average of several fields taken. This average number multiplied by 5000 gives the number of bacteria in the original 0.01 c.c. of milk. The total bacteria count of the milk is obtained by multiplying this result by 100, the original dilution.

**Intestinal Bacteria in Milk.**—The presence of intestinal bacteria, chiefly *Bacillus coli* and *Bacillus lactis aërogenes*, may be determined by the use of plates of lactose litmus agar or Drigalski-Conradi medium upon which the acid-producing organisms produce red colonies. Or the intestinal bacteria may be obtained by the use of lactose fermentation tubes planted with small quantities, 1 to 5 c.c. Plates should be made from the tubes showing positive fermentation and the organisms identified by the usual methods. The count of intestinal bacteria on plates is occasionally valuable for determining the extent of intestinal contamination of the milk. This may be of considerable sanitary significance.

**Spore-bearing Organisms in Milk.**—Resistant aërobic spore-bearing organisms are frequently present on plates made from fresh milk. In older samples these organisms do not usually appear, being overgrown by the lactic acid streptococci and the intestinal bacteria. They may easily be obtained by heating samples of milk to 80° to 85° C. one-half to one hour and pouring plates immediately or after incubation of twenty-four hours. The organisms which are found by this method are derived from the spores present in the milk. A great many different types of spore-bearing bacteria have been described from this source. The predominant species are *Bacillus subtilis*, *Bacillus cereus*, *Bacillus albolactis*.

**Anaërobes in Milk.**—Anaërobic species vegetating in raw milk are difficult to demonstrate since they are usually overgrown on plates by the facultative anaërobes. An approximate idea of the obligate anaërobes presumably present may be gained by heating the milk and making plates by anaërobic methods. The anaërobes thus obtained represent spore material. The chief anaërobes found in this way are *Bacillus tertius*, *Bacillus sporogenes*, *Bacillus Welchii*, and *Bacillus bifermentans* (Bliss[2]).

**Pathogenic Organisms in Milk.**—On the basis of epidemiological evidence we know that cow's milk is a common vehicle for the transmission of a number of important diseases such as typhoid fever and Asiatic cholera, intestinal tuberculosis, especially in children, foot-and-mouth disease, and streptococcus angina, while it may rarely serve to convey paratyphoid fever and diphtheria. It was formerly regarded as an important agent in the spread of scarlet fever,

but the extent to which it conveys this disease is debatable in view of our recently acquired knowledge as to the etiology of this infection. The actual number of disease-producing organisms which have been isolated from milk is much lower than is suggested by epidemiological studies, despite the fact that milk is a favorable medium for the growth of many of our most virulent pathogens. It is possible that the pathogens die out in competition with the ordinary milk bacteria, and again that our methods fail to reveal them even when present.

*Tuberculosis.*—The bovine tubercle bacillus is present in cow's milk reagularly when the udder of the animal is diseased. The number of these organisms depends upon the extent to which open ulceration occurs in the gland. With disease in other parts of the body tubercle bacilli are only occasionally discharged with the milk. According to the Royal British Tuberculosis Commission animals injected subcutaneously with tubercle bacilli may begin to discharge them into milk within seven days and continue to do so until their death. With small healed foci of disease tubercle bacilli are seldom found in the milk. In active tuberculosis, especially when the lungs of the animal are affected and a good deal of material is coughed up, the milk may be contaminated mechanically with the organism, since it may be widely distributed over the udder and flanks and about the stable. The tubercle bacilli may also be swallowed and be present in the dejecta, which thus serves as another possible source for the organism.

Milk may be contaminated occasionally by the human tubercle bacillus when active cases of tuberculosis exist among the people who handle the milk. Such contamination is, of course, always mechanical, and is more apt to occur when proper methods of handling the milk are not in vogue.

The presence of tubercle bacilli in milk may be determined by centrifugalizing small samples and staining the sediment by acid-fast methods. Non-pathogenic acid-fast organisms are occasionally found and a more accurate method of determining the presence of genuine tubercle bacilli is by the inoculation of susceptible animals like rabbits and guinea-pigs with measured quantities. Friis[3] estimated that tubercle bacilli were present in market milk in Germany in 14.3 per cent. of 28 specimens. Hope found them in 5.2 per cent. of 228 samples, and Massone, in 9 per cent. In America the market milk of large cities not infrequently contains virulent tubercle bacilli. Anderson[4] found that 6.72 per cent. of the samples in Washington in 1907–08 contained enough tubercle bacilli to kill guinea-pigs in 1 c.c. samples.

In *typhoid fever* milk, next to water, is the chief agent of transmission among objects in the external world. Trask[5] has collected some 179 epidemics of typhoid fever due to milk contaminated by the typhoid bacillus and a number of other epidemics are on record since Trask's report. The isolation of the typhoid bacillus from milk is very rare despite the fact that milk is a favorable medium for the growth of the organism. Davis believes that the lactic acid developing from the growth of streptococci in milk may inhibit and destroy the typhoid bacillus. The organism has been isolated by Konrádi[6] from the milk of a bakery where a number of people with typhoid fever had been in the habit of purchasing milk. According to Reynolds[7] the typhoid bacillus has been obtained from market milk in Chicago three times in routine examinations covering a period of eight years.

While *streptococci* are normally present in milk, many of these organisms are quite without pathogenic properties. In the disease of the udder known as mammitis or "garget" pathogenic streptococci may be discharged into

milk along with pus-cells and red blood-corpuscles. Such organisms are often virulent to animals and are presumably pathogenic to man. Occasionally highly virulent streptococci are distributed by milk and give rise to severe epidemics of disease in man with a high mortality. Such epidemics are usually known as "streptococcus sore throat." They have been reported especially by Capps and Miller,[8] Hamburger,[9] and Winslow.[10] The streptococci giving rise to this type of infection are virulent to animals and produce a peculiar type of hemolysis on blood-agar plates, according to Smith and Brown.[11] The source of such streptococci in milk is not entirely clear, but apparently they are of human rather than of bovine origin.

A number of epidemics of *diphtheria* have been traced to milk and 23 have been collected by Trask. The demonstration of the diphtheria bacillus in milk is difficult since non-virulent diphtheroids are occasionally present. Two definite isolations of the diphtheria bacillus from milk have been reported by Dean and Todd.[12] In both of these the suspected organisms were carefully tested for virulence and toxin-producing power and found to conform in all particulars to the Klebs-Loeffler bacillus. Other isolations have been reported by Bowhill and by Eyre.[13]

Goat's milk is not infrequently contaminated by *Bacillus melitensis*, the etiological agent in Malta or Mediterranean fever. The English Commission found that about 40 to 50 per cent. of the goats on the Island of Malta were infected by this organism and a considerable number of these infected animals (10 per cent.) distributed the organism in their milk even without manifest disease of the udder. A few cases of Malta fever have been reported in America, chiefly from Texas, where the disease is known as "goat fever" and is attributed to the use of raw milk from goats imported from the Mediterranean a number of years previously. An organism closely related to *Bacillus melitensis*, *Bacillus abortus* of Bang, is frequently present in cow's milk in America as shown by Fabyan.[14] While this organism may be distributed by milk and cause disease in cattle, its virulence for man has not thus far been established definitely.

**Changes in Raw Milk on Aging.**—Under normal circumstances the bacteria present in milk multiply rapidly, their growth depending almost directly upon the temperature at which the milk is kept. The different species do not multiply at the same rate, but certain types like the streptococci and the intestinal bacteria which act on lactose develop at the expense of the other species. In consequence, milk sours and clots from the lactic acid produced. Just before the actual precipitation of the casein the number of bacteria is very high, often running up to 3,000,000 to 4,000,000 per cubic centimeter. The bacterial count in milk, therefore, bear a direct relation to the temperaature at which the milk is stored and the period of time which has elapsed between the collection of the sample and the period at which the count is taken. The counts are higher in summer than in winter. Old milk always has a higher bacterial count than fresh milk, but it should be remembered that the organisms present in old milk are practically always harmless species like the lactic acid streptococci. When the initial contamination of the milk is excessive, the milk turns sour and clots earlier than when the initial contamination is less marked. In general the aërobic and anaërobic spore-bearing bacteria are inhibited by the growth of the lactic acid organisms. In very old milk many of the organisms are killed off by the high acidity, and such milk may actually putrefy from the growth of proteolytic bacteria. In such samples of old milk there is frequently a surface growth of pigmented bacteria and molds.

**Effect of Heat on the Bacteria in Milk.**—By heating milk to temperatures

between 50° and 60° C. for one-half to one hour it is possible to destroy a large number of the vegetative organisms. To what extent the lactic acid streptococci are killed by this amount of heat is not clear, since Ayers, Johnson, and Davis[15] have shown that certain of these streptococci withstand this temperature. Pathogenic organisms like the typhoid bacillus and the cholera vibrio, pathogenic streptococci, etc., are practically always destroyed. The tubercle bacillus is usually somewhat more resistant, but it has been proved by Smith[16] that a temperature of 60° C. maintained for fifteen to twenty minutes is sufficient to destroy this species, provided the heat is so applied as to reach the organisms. The spores of the resistant aërobic bacteria, like *Bacillus subtilis*, and of the anaërobes, like *Bacillus Welchii* and *Bacillus sporogenes*, are not destroyed at this temperature. In consequence milk heated sufficiently to destroy vegetative bacteria and then allowed to age always putrifies. Sometimes the so-called stormy fermentation occurs from the multiplication of *Bacillus Welchii* and *Bacillus sporogenes*, a reaction characterized by clotting, gas production, retraction and shrinking of the clot, with an occasional liquefaction. Usually considerable amounts of butyric acid with a characteristic odor are produced. In other instances the proteins in the milk are rapidly coagulated and then dissolved, this being due to such organisms as *Bacillus albolactis*. Finally, a rapid liquefaction of the milk protein without preliminary coagulation may occur. This is generally due to such forms as *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus megatherium*. Very old milk which has decomposed from the growth of these resistant aërobes and anaërobes may show the presence of protein degradation products like indol, methyl mercaptan, hydrogen sulphid, etc. The destruction of the spores of these resistant bacteria in milk is somewhat difficult. Flügge[17] has shown, for instance, that some of them withstand boiling for long periods, six to eight hours. Such spores are easily destroyed by temperatures above the boiling-point. Milk may thus be sterilized by heating in the autoclave for short periods, ten to fifteen minutes, at a pressure of 15 to 20 pounds.

**Milk Poisoning.**—Poisoning from raw milk is very uncommon. The milk from cattle suffering from foot-and-mouth disease is said to be poisonous on ingestion, but this milk always contains the etiological agent of the disease which is virulent to man. The rare affliction known as "milk sickness," which was formerly somewhat common in the outlying portions of the Middle West, is due to the use of milk from animals suffering from "trembles." The etiology of trembles is still somewhat obscure. It is undoubtedly due to the grazing of cattle in areas where poisonous plants grow abundantly, probably some representatives of the euphorbiaceæ. The milk from animals with trembles apparently contains the poisons responsible for the condition. Its use is followed by "milk sickness," a disease characterized by disturbances of the alimentary canal, violent headache, and nervous manifestations. The mortality is not high. A chronic form of the disease known as "slows" not infrequently develops, with obstinate constipation, lack of appetite, great muscular weakness, and trembling on exertion.

Milk may possibly be poisonous from the presence of bacterial toxins. This condition is known as "galactotoxismus" and cases have been reported by Vaughan and Wheeler. Galactotoxismus was formerly attributed to the ingestion of milk in which poisons were evolved as a result of bacterial decomposition. At the present time most cases of milk poisoning of this type are regarded as cases of infection from the paratyphoid bacillus, *Bacillus enteritidis* of Gärtner, etc. Whether decomposed milk as such is poisonous to man on ingestion is not clear. It usually has a disagreeable

odor and a bitter taste and should not be used in any quantity. It has been shown by Ford and Lawrence[18] that heated milk allowed to age and decompose from the growth of the resistant spore-bearing bacteria may contain the toxin of *Bacillus Welchii*.

**Abnormal Changes in Milk.**—Milk is occasionally contaminated by organisms which bring about peculiar abnormal changes. The growth of *Bacillus lactis erythrogenes* of Hueppe and Grotenfeldt or closely related organisms producing a red pigment may turn the milk red, and the same change may be due to micrococci and yeasts which produce a red coloring-matter. The growth of *Bacillus cyanogenes* turns milk green or greenish-yellow. Milk is rarely slimy from the development of *Bacillus lactis viscosus*. Ward thinks that this organism is derived from contaminated water. A bitter taste in milk is usually due to the growth of organisms producing butyric acid. According to Conn[19] certain diplococci can produce this taste. Harrison has reported bitter milk from a yeast, *Torula amon*. Occasionally alcoholic fermentation occurs from the multiplication of yeasts which attack the lactose and produce alcohol. Alcoholic fermentation is employed in the Orient for the manufacture of beverages from milk, chiefly mare's milk.

**Pasteurization of Milk.**—In order to destroy pathogenic organisms in milk and enhance its keeping qualities by the destruction of excessive numbers of bacteria, the process known as pasteurization is now widely employed. The principle of pasteurization is the heating of milk for brief periods to a temperature high enough to destroy the vegetative bacteria, usually 60° C. for twenty to thirty minutes, 65° C. for ten to fifteen minutes, 70° C. for one to two minutes. The older methods of flash pasteurization, in which milk was heated for two to three minutes to high temperatures, 70° to 80° C., have now been shown to have little value. At the present time pasteurization is carried out by the holding method, the milk being heated in large resceptacles or vats for twenty to thirty minutes to temperatures in the neighborhood of 60° C. The regulations for pasteurization differ in different communities. In general, municipal ordinances governing pasteurization require milk to be heated to 142° to 145° F. for thirty minutes (61+° to 62+° C.). This temperature suffices to kill ordinary vegetative bacteria and is just sufficient to destroy the tubercle bacillus. Pasteurization at this temperature is thus slightly above the borderline of safety. Pasteurization at higher temperatures, 158° F. (70° C.), which would theoretically ensure the destruction of pathogenic organisms like the tubercle bacillus in milk, has great commercial disadvantages in that it destroys the cream line and is apt to impart a cooked taste. For practical purposes pasteurization for thirty minutes at 142° to 145° C. (61+° to 62+° C.) can be relied on to destroy any pathogenic bacteria which may have found their way into milk. Abundant experience in large cities where pasteurization is carried out in accordance with municipal regulations has proved that infectious diseases like typhoid fever, streptococcus sore throat, and tuberculosis are not transmitted by milk pasteurized at this temperature for this period of time.

**Boiled Milk.**—In order to destroy the pathogenic organisms with certainty milk may be boiled for brief periods, five to ten minutes. The use of boiled milk has been practiced widely in Europe, especially in Germany, but has never made much headway in America. The chief disadvantages are that boiling imparts a decided taste to milk, which is offensive to many people, and tends to destroy the cream line so that the supply of cream is not sufficient for household needs. Certain of the proteins in milk are coagulated by boiling, and many if not all the ferments and immune substances are destroyed. The nutritive properties of milk are not materially injured by

boiling, and in emergencies, where a safe supply of pasteurized milk is not available, boiling should be insisted on. Rarely scurvy in children has been attributed to the use of boiled milk. Such an exceptional condition is readily avoided by adding a little lemon or orange juice to the child's diet.

**The Grading of Milk.**—The dependence of the number of bacteria in milk upon its initial contamination, upon the methods of handling, upon the temperature at which it is kept, and the period of storage between its collection and use has demonstrated that milk counts may be employed as a public health measure to regulate milk supplies, especially in large cities. With proper care in the collection of the milk and its storage at low temperatures the bacterial counts may be kept very low. Where proper methods for ensuring cleanliness are not carried out and the milk is kept for long periods at high temperatures, the counts may be very high, 100,000 to 500,000 bacteria per cubic centimeter. For this reason milk is now frequently graded according to standards established by cities or states. These standards differ in different communities. In New York City the following grades are established (Park and Williams[20]):

Grade A milk should come from cows free from tuberculosis as determined by the tuberculin test and contain no more than 60,000 bacteria per cubic centimeter. It may be raw or pasteurized. Grade B milk should contain not more than 1,500,000 bacteria per cubic centimeter before pasteurization, and not more than 50,000 bacteria per cubic centimeter after pasteurization. It must be pasteurized before selling. Grade C milk must contain no more than 100,000 bacteria per cubic centimeter after pasteurization, but no special limit is placed upon the number of bacteria in the milk before pasteurization. Grade C milk is supposed to be used only for cooking purposes. The pasteurization must be carried out by the holding method for thirty minutes at 142° to 145° F. At the same time that the bacterial counts are made to determine the quality of the milk conditions at the dairy farm and at the various milk stations are examined by inspectors, proper cleanliness is enforced, and individuals suffering from infectious diseases like typhoid fever, where the organisms may be carried by milk, are not permitted to handle it.

**Selected Milk.**—Milk collected under rigid precautions to ensure its cleanliness, obtained from herds free from tuberculosis as shown by tuberculin tests, handled only by persons free from infectious disease, and showing a low bacterial count, is sold in many cities in America as *selected milk*. The bacterial count of such selected milk when fresh is frequently very low, often less than 5000 bacteria per cubic centimeter. In general, the precautions which must be followed in preparing selected milk add materially to its cost. Such milk is, therefore, only available for the well-to-do classes and is not usually needed except with children with poor digestions, who may not thrive on pasteurized milk. In the use of selected milk it must always be borne in mind that its freedom from the agents of infectious disease is not guaranteed by pasteurization. An unreported case of typhoid fever or a typhoid carrier among the personnel thus exposes the milk to danger. Occasionally selected milk with a low bacterial count may serve as a vehicle for the transmission of typhoid fever.

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## BACTERIA IN MILK PRODUCTS

Cream usually has a higher bacterial count than whole milk and the types of bacteria in it are identical with those found in the milk itself. Ice-cream, which is theoretically frozen cream, contains, in reality, many other ingredients, gelatin, fruit juices, etc. It usually has a high bacterial count, since the organisms in it are derived from the milk and are not injured by the procedures adopted in its manufacture. The number of bacteria is much lower when the ice-cream is prepared from pasteurized milk. Ice-cream is popularly supposed to be a vehicle for the transmission of diseases like typhoid fever, but scientific evidence to support this view is by no means satisfactory.

Butter is obtained from milk by churning and consists of the fat globules of the cream. The cream must be allowed to ripen in order to make butter of the proper flavor. During this ripening the bacteria in the cream increase rapidly, running up to many millions in number. Conn[1] attributes the ripening to the multiplication of the lactic acid bacteria. Butter rarely contains the tubercle bacillus, but this organism has been found in market butter by several observers, notably Obermüller,[2] Rabinovitch,[3] Boyce,[4] Woodhead,[5] and others. Under experimental conditions tubercle bacilli may remain alive for some months in butter kept at the temperature of the refrigerator (Mohler[6]). The acid-fast organisms usually found in butter are non-pathogenic saprophytes like the butter bacillus of Rabinovitch. Butter may occasionally become rancid from the bacterial decomposition of the fats, developing a disagreeable odor and taste. This change is usually due to multiplication of bacteria producing butyric acid. Such butter does not act as a vehicle for the transmission of disease, but cannot be marketed. Rancid butter is frequently cleaned and renovated and the pure fats drawn off. It is then often sold as fresh butter.

Cheese is obtained from milk products by various processes of protein decomposition, and contains a variety of micro-organisms, chiefly molds, yeasts, spore-bearing bacteria, and lactic acid streptococci. Pathogenic bacteria, which may happen to find their way into the milk from which the cheese is made, probably do not survive the bacterial changes occurring in its manufacture. According to Harrison and Galtier, however, both human and bovine tubercle bacilli may rarely be present in cheese. Cheese made from goat's milk containing excessive numbers of *Bacillus melitensis* may possibly harbor this organism.

*Buttermilk.*—Several different types of bacteria produce excessive

amounts of lactic acid from milk and various beverages have been manufactured by the use of pure cultures of these organisms. Some of these are Bulgarian buttermilk made by fermenting milk with *Bacillus bulgaricus*, and koumys made from milk by the use of certain lactic acid bacteria and yeasts. Ordinary buttermilk as prepared on farms is usually whole milk soured by the acid produced by the ordinary lactic acid streptococci. Other commercial preparations are Maadsoun and Zoolac. All such buttermilks contain large amounts of lactic acid and excessive numbers of the particular lactic acid bacteria used in their preparation. The use of sour milks of these types, especially the Bulgarian buttermilk, has been advocated by Metchnikoff to restrain excessive intestinal putrefaction (see chapter on Bacteriology of Intestines).

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## BACTERIA IN SHELL-FISH AND FISH

Oysters, clams, and mussels contain but few bacteria under normal conditions. The organisms are chiefly derived from the water in which the shell-fish have developed or in which they are deposited just before consumption. Oysters and mussels are not infrequently the vehicles for the transmission of typhoid fever. The classic epidemic of typhoid fever in Middletown, Connecticut, reported by Conn,[1] in which 9 students got typhoid fever from oysters fattened in a sewage-polluted stream, first indicated clearly the danger to man of this type of bacterial contamination of oysters. Buchan[2] and Newsholme[3] have likewise shown that a certain amount of typhoid fever in England is due to the use of mussels grown in sewage-polluted waters. Oysters may also be contaminated by the typhoid bacillus in the process of shucking and canning, either by incipient cases of the disease or by carriers. The juice of shell-fish is an excellent medium for the growth of *Bacillus typhosus*. Actual isolations of the typhoid bacillus from oysters, clams, etc., are very rare. Shell-fish grown in sewage-polluted water containing *Bacillus coli* frequently harbor this organism and its presence is employed as an index of pollution. Standard methods have now been agreed upon by the American Public Health Association for the determination of the sanitary quality of oysters. The shells are opened carefully under aseptic precautions and the juice drawn off in sterile pipets. The juice of 5 oysters is mixed and a bacteriological count made. The juice of each one of 5 oysters is planted in lactose fermentation tubes in 1 c.c., 0.1 c.c., and 0.01 c.c. quantities. After three days' incubation at 37° C. the development of over 10 per cent. of gas in the closed arm of any tube is considered as a positive presumptive test for the presence of *Bacillus coli*. The oysters are now "scored." A positive reaction in a fermentation tube inoculated with 1 c.c. is recorded as 1, a positive reaction in a tube inoculated with 0.1 c.c. is recorded as 10, and a similar reaction in a tube inoculated with 0.01 c.c. quantities as 100. The score is the sum of these recorded numbers. The presumptive tests ahould be confirmed by the isolation of *Bacillus coli* from the lactose tubes giving a positive fermentation. For the scoring of shucked oysters the fluid from well-mixed samples is planted in triplicate in lactose fermentation tubes in 1 c.c., 0.1 c.c., 0.01 c.c., and 0.001 c.c.

quantities. The score is determined in the same way except that the average is obtained by adding the results and dividing by 3. No standard score for shucked oysters has been agreed upon, but the United States Pure Food Board recommends the condemnation of shucked oysters in which a score of 32 or over has been obtained from the juice. According to Wells,[4] Pease,[5] Park and Williams[6] and others polluted oysters placed in pure water gradually purify themselves. Gorham[7] has shown that oysters contain fewer bacteria in winter than in summer and believes that oysters hibernate in cold weather. In general, the bacterial count of both raw and shucked oysters is higher in summer than in winter.

Shell-fish decompose rapidly if kept in a warm place. In oysters there is a liquefaction of the protein materials and a peculiar odor develops. Apparently this change is due almost entirely to the aërobic species originally present. Recent observations by Eliot[8] indicate that spoilage is due to streptococci, members of the coli-aërogenes group, pigmented organisms and vibrios, anaërobic species not playing any important rôle in the decomposition. Old decomposed oysters may be intensely poisonous to man on ingestion. Brosch[9] has reported the death of a man in twelve hours after eating badly decomposed oysters. Decomposed mussels are occasionally the cause of disease in man, the condition being known as "mytilotoxismus." The first instances of this type of poisoning were reported by Schmidtman and Virchow.[10] Here several persons who had eaten mussels, *Mytilus edulis*, collected from sewage-polluted waters, were taken sick in a few hours with intense gastro-intestinal and nervous symptoms. Three of these people died in a few days. This condition was regarded as an intoxication from the shell-fish, decomposed as a result of excessive bacterial contamination. We have no definite knowledge of the type of bacteria which may bring about a decomposition in oysters or mussels as a result of which they become poisonous to man on ingestion.

Fish also decompose rapidly at high temperatures and decomposed fish are extremely poisonous to some individuals. The cause of this decomposition is still obscure. In general, illness from decomposed fish is now regarded as infection from organisms of the paratyphoid group with which the fish may happen to be contaminated. In countries where fish are eaten raw in large quantities, as in Japan, they may be an important factor in the spread of typhoid fever. A thorough study of the decomposition of fish, especially salmon, has been made by Hunter[11] who found the same types of organisms in the decomposing muscles as in the water from which the fish were taken. He believed that a count of 15,000,000 per gram was sufficient to explain the spoilage. The total bacterial count was of value in judging the quality of salmon only when the salmon were not feeding when caught and the alimentary tract was devoid of food.

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## BACTERIA IN FOODS

Foods of nearly every variety are contaminated under natural conditions with bacteria from the soil, air, and dust, from the water in which they are washed and prepared for cooking, and from the hands of the individuals who handle them in the markets and in the household. As a general rule the bacteria on foods are without pathogenic action when taken into the alimentary canal of man and the majority are destroyed on cooking. How far food is actually sterilized by cooking is not clear. Food may apparently be a vehicle for the transmission of both typhoid fever and paratyphoid fever and Sawyer has shown experimentally that micro-organisms like the typhoid bacillus inside sticks of macaroni are not destroyed by ordinary cooking. Raw foods, fruits, and vegetables may convey Asiatic cholera. The source of this contamination is probably the water in which they are washed. They may also be polluted by the hands of those who have a mild type of the disease or who are temporary carriers. Raw foods also apparently play a rôle in the spread of typhoid fever in countries like China where enteric fever is widely prevalent in certain districts. How far raw foods may continue to harbor organisms like the typhoid and paratyphoid bacilli has not been determined. It is of interest to note that raw fruits and vegetables grown on sewage farms, where large quantities of municipal sewage is received for treatment, are frequently marketed. No epidemics of typhoid fever have thus far been attributed to their use, when the sewage farms on which they are grown are under proper control.

**Canned Goods.**—In commercial canning of fruits, vegetables, and meats all the bacteria are usually destroyed except the resistant spores of certain species. When these are the spores of the non-pathogenic aërobes and anaërobes little harm results except that the food is liable to spoil and become unfit for consumption. Sometimes the material canned contains the spores of *Bacillus botulinus*, the multiplication of which gives rise to a toxin, the only bacterial toxin known which exerts its harmful effects when taken into the alimentary canal. Meats, fruits, and vegetables may harbor the spores of *Bacillus botulinus* and in America botulism has been produced by commercially canned goods, chiefly in the case of ripe olives and canned spinach. With home-canned goods it has been reported from canned spinach and asparagus. In Europe botulism has been traced principally to canned meats, but a few cases are on record from canned vegetables. It is still a question whether canned goods in which *Bacillus botulinus* has multiplied and produced its toxin exhibit evidence of spoilage. In the majority of instances the food has a disagreeable odor, but even when this is present enough material may be consumed inadvertently to bring on fatal consequences. The only absolute safeguard against botulism is the complete sterilization of the material which is used for canning, preferably in the container. At the same time precautions should be taken so that rotten decomposed materials are not to be canned and used as food under any circumstances. The extent to which decomposed food material is injurious to man apart from the contamination by *Bacillus botulinus* or by members of the paratyphoid-enteritidis group is still unsettled. The present tendency in America is to explain food poisoning on the theory that the cases are really paratyphoid fever or similar infections.

**Meats.**—Raw meats from healthy animals may be contaminated by bacteria from many sources, chiefly the intestinal discharges of the animals killed, the utensils used in preparation and storage and the hands of the workers. At the same time the bacteria of the air and the dust may fall upon meat as it is stored in the shops and offered for sale. The bacteriological

examination of market meat reveals the presence of pigmented air organisms, spore-bearing aërobes and anaërobes, yeasts and molds. As meat is kept, especially during warm weather, the bacteria develop and eventually render it foul and putrid. Tissier and Martelly[1] have shown that the important organisms responsible for the spoilage of meat are the proteolytic aërobes and anaërobes, principally *Bacillus perfringens* (*Bacillus Welchii*). Recent investigations by Hofstadt[2] indicate that the decomposition in meat is due primarily to five species of anaërobes, the aërobes playing but a subsidiary part. The anaërobes isolated by Hofstadt consisted principally of *Bacillus Welchii* of Welch and Nuttall, *Bacillus sporogenes* of Metchnikoff, *Bacillus tertius* of Henry, *Bacillus bellonensis* of Sacquéppé, and *Bacillus aërofœtidus* of Weinberg and Séguin. The final changes in spoilage are probably due to molds.

Attempts to determine a relation between the numbers of bacteria in samples of meat and the extent of spoilage have not given uniform results. Marxer,[3] who studied Hamburger steak, suggested 1,000,000 bacteria per gram as a possible standard. Later, Weinzirl and Newton,[4] investigating Hamburger steak in Seattle, Washington, and comparing their bacterial counts with organoleptic and ammoniacal tests,* came to the conclusion that the Marxer standard was too low and proposed a standard of 10,000,000 bacteria per gram. Recently Hofstadt[5] on the basis of meat examinations in Baltimore, in which she has compared the bacterial counts with the amount of spoilage, has been unable to find any correlation between the number of aërobic bacteria present and the degree of decomposition. In her opinion the presence of proteolytic anaërobes may be used as a rough index of the extent to which meat is contaminated and likely to spoil with age and suitable temperatures.

Meat from animals suffering from infectious diseases may harbor the agents of these infections. The muscle and fat of the animals, both of which are included in "meat," are, however, generally not attacked by the disease process. They become contaminated accidentally from diseased lungs and glands or from the excreta. With certain infections like tuberculosis the organisms do not find the proper conditions for development in raw meat and in consequence meat is seldom a factor in its spread. With other infections, chiefly those now grouped as the "paratyphoid infections," the organisms are able to multiply in or on the meat which thus becomes a vehicle of transmission. Rarely also the organisms of this group invade the bloodstream of the animal before death and the meat already contains them at the time the animals are killed. The principal pathogenic bacteria of this group are *Bacillus enteritidis* of Gärtner (the Aertrycke bacillus) and *Bacillus suipestifer* (the hog-cholera bacillus). In this connection it is important to note that toxic substances develop in cultures of *Bacillus paratyphosus* B as has been shown especially by Ecker.[6] Koser[7] and Geiger, Benson, and Davis[8] have found that members of the paratyphoid-enteritidis group are able to multiply in various food stuffs, including vegetables and meats. Meat may also be contaminated accidentally by the spores of *Bacillus botulinus* and give rise to botulism as in the original case of van Ermengem. The carcasses of rabbits dead of tularemia are heavily infected by *Bacillus tularense* and this infection may be carried to man mechanically. Finally meat may become infected by cholera vibrios, typhoid bacilli, or human paratyphoids during handling, either by actual cases of the disease or by carriers.

* The organoleptic test consists of smelling and tasting meat. Meat with a foul odor and disagreeable taste is said to give a positive organoleptic test.

Practically all pathogenic bacteria likely to be present in meat are destroyed by thorough cooking except *Bacillus botulinus* and its toxin. It is not yet certain whether decomposed meat can give rise to serious illness in man apart from infection by specific organisms. As in the case of other foods the present tendency is to explain all cases of meat poisoning except botulism as instances of infection by organisms of the typhoid or paratyphoid group. The use of decomposed meat even after thorough cooking should be avoided on general hygienic principles since many individuals are undoubtedly poisoned by it with mild gastro-intestinal symptoms.

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# PART IV

# INFECTION AND IMMUNITY

## Chapter I

## PRINCIPLES OF INFECTION

### GENERAL PROPERTIES OF INFECTIOUS AGENTS

To indicate the penetration of a viable agent from outside into the body where it increases and produces disease we employ the term *infection* (L. *inficere*, to spoil or to pollute). The term should be limited, as von Behring[1] first suggested, to the action of a viable agent. The disease caused by the product of this viable agent is not properly an infection, but an *intoxication*. In most instances the substances causing the latter originate in the body, not in the external world. A few exceptions to this rule have been observed, notably in such food intoxications as botulism, where the poisonous substance is found in the food contaminated by *Bacillus botulinus*.

The infectious agent comes originally from outside the body under all conditions, even in the so-called auto-intoxications, where disease may come from micro-organisms which have been in the body for years. The tissues and body cavities of man and animals are sterile under normal conditions in intra-uterine life, the bacteria getting in only after birth.

In consequence of the fundamental work of Cohn, Pasteur, and Koch it has now been established that infectious diseases are due to definite infectious agents, and can be produced only by these agents. We express this by saying that an infectious agent is specific for a disease. Thus *Bacillus typhosus* causes only typhoid fever, not tuberculosis or anthrax, and in every case of typhoid fever the typhoid bacillus is present.

For the development of an infection it is first necessary that the specific infectious agent penetrate the tissues of the body and find there favorable conditions for further multiplication. We no longer believe that the infectious material must ripen outside the body, as Pettenkofer taught, in order to give rise to other cases of disease. In the last analysis the etiological agents of disease have probably always been derived more or less directly from previously infected animals or man, although they may multiply in the external world. They may be carried by air, water, foods, or indeed by any objects to which they can cling for any appreciable time.

The micro-organisms which can increase in the living tissues of man and animals and produce disease are called *infectious*, *parasitic*, or *pathogenic*, in contrast with the many species found in nature that do not possess this property and are called *saprophytic*. The pathogenic agents must actually penetrate the cells in all cases. As long as they merely live on the surface of the body they do not cause infection. Pathogenic organisms like staphylococci, streptococci, pneumococci, and sometimes the diphtheria bacillus may be present on the mucous membranes of the body cavities which communicate with the atmosphere, as the nose and throat, without causing illness in the individual. Similarly the mere presence of pathogenic bacteria on the outer integument does not give rise to disease. Thus Wigura[2] has found virulent tubercle bacilli on the hands of attendants in an institution

for the care of the tuberculous; typhoid bacilli have been cultivated from the hands. These persons did not have either tuberculosis or typhoid fever. Pathogenic organisms are frequently present on the mucous membranes of people who come into close and intimate contact with cases of infectious disease. Thus in periods when cerebrospinal meningitis is epidemic a certain number of people harbor the meningococcus in the nose or throat. In the early investigation of Flügge[3] it was estimated that this number might be as high as 70 per cent. More recently it has been shown that true meningococci are found in only a small proportion of the individuals who come in contact with cases of meningitis, 10 to 20 per cent. according to Bruhns and Hohn.[4] In other instances normal people may harbor the infectious agent without acquiring the disease. Pfeiffer,[5] states that 18 to 22 per cent. of the population may have the cholera vibrio in their dejecta in times of epidemics, while Frosch[6] has found the typhoid bacillus in the stools of contacts in 5 per cent. of the cases. The diphtheria bacillus has been especially investigated in this connection. Loeffler[7] finds that 10 to 20 per cent. of those in the neighborhood of patients with diphtheria have virulent diphtheria bacilli in the nose and throat. Doull and Fales[8] have recently examined combined nose and throat cultures from 7790 children in Baltimore, selecting the cases at random from schools situated in wards of the city where diphtheria was prevalent. They found 409 carriers of organisms with the morphology of the diphtheria bacillus (5.25 per cent.) and 136 carriers of virulent diphtheria bacilli (1.75 per cent.).

In order to cause an infection the pathogenic bacteria must get into the tissues, either through wounds or defects in the skin or mucous membranes, or through normal uninjured structures. It becomes a matter of great importance therefore to determine how far the uninjured skin or mucous membrane offers a complete protection against the entrance of infectious agents. The degree of protection afforded by the *skin* depends almost entirely upon the type of organism. With some species like the typhoid bacillus and the tubercle bacillus, the uninjured integument of adults offers a complete protection; the same holds true for the spores of the tetanus bacillus and other pathogenic anaërobes. In bubonic plague minute invisible injuries of the human skin, such as are made by the bite of a flea, permit the entrance of the plague bacillus. In animals plague bacilli can apparently pass through the uninjured integument, as in guinea-pigs, where plague can be produced by rubbing cultures on the shaved uninjured skin of the abdomen. The ease of penetration of the human skin by pathogenic bacteria depends also upon other factors, such as age and sex. According to Cornet, the skin of women is more easily penetrated by the tubercle bacillus than that of men; the skin of children more easily than that of adults. Thus we have more tuberculous infection of the skin and the subjacent glands in women and children than in men.

The uninjured *mucous membrane* offers far less protection against pathogenic organisms than the uninjured integument, but here also much depends upon the type. The normal mucous membrane of the alimentary canal affords complete protection against the tetanus bacillus in both animals and man. Horses, most sensitive to tetanus, may harbor *Bacillus tetani* in the contents of the bowel without developing tetanus and apparently the same condition holds true for human beings (TenBroeck and Bauer). The intestinal mucosa under normal conditions is usually a complete bar to the entrance of such organisms as staphylococci, streptococci, and colon bacilli. Bacteria like the typhoid bacillus and the cholera vibrio can evidently penetrate the uninjured mucosa of the intestines, and the same con-

dition apparently holds true for the tubercle bacillus. A number of authors, notably von Behring,[9] Calmette and Guérin,[10] and Vallée,[11] have shown that tuberculosis of the lungs can be produced by feeding material containing the tubercle bacillus. Here the organisms apparently pass through the uninjured intestinal mucosa with the chyle into the mesenteric glands, then up the ductus choledicus into the subclavian veins, and finally through the right heart and the pulmonary artery into the capillary vessels of the lungs.

The respiratory mucosa offers little protection under normal conditions to the invasion of bacteria, but again this depends somewhat on the type of organism. Thus it has been shown by Cornet,[12] Laschtschenko,[13] Heyman,[14] and others, that the tubercle bacillus can invade the uninjured respiratory mucosa. Flügge[15] observed that it was possible to render a guinea-pig tuberculous by inhalation with a dose only 1/2500 part of the amount necessary to lead to local tuberculosis of the mesenteric glands. Laffert claims that severe inhalation tuberculosis can be caused by 1/2,000,000 part of the quantity of tubercle bacilli which can surely produce tuberculosis from the intestinal tract.

The normal conjunctiva of the uninjured eye is easily penetrated by certain bacteria. Thus the conjunctiva of the newborn child is especially prone to infection by the gonococcus, as in *ophthalmia neonatorum*. Apparently the same condition holds true in adult gonorrheal ophthalmia, but here possibly minute injuries are present such as those resulting from vigorous rubbing of the eye. In bubonic plague it has been shown by Kolle[16] and others that after very slight injuries of the ocular conjunctiva plague can be induced in rats by merely dropping cultures of *Bacillus pestis* on it. Tularemia apparently follows the introduction of only a small amount of infectious material into the normal eye.

## PORTAL OF ENTRY

With certain infectious agents disease cannot be produced indiscriminately in any region of the body, rather must they enter through some particular area which is known as the *portal of entry*. For example, the typhoid bacillus can cause typhoid fever, and the cholera vibrio cholera only when the etiological organisms enter the body through the intestinal tract, *i. e.*, by means of the stomach. The experiments of Ferran, who employed living vibrios for vaccination against cholera, demonstrated this principle conclusively for this disease, since not a single case followed the introduction of the vibrio by this route. The tetanus bacillus again apparently never produces tetanus from the alimentary canal, but requires for its portal of entry some injury to the skin and subcutaneous tissues.

With other bacteria disease may follow wherever the organisms enter the body. The tubercle bacillus can bring about tuberculosis from the skin, intestine, respiratory mucosa, and the urogenital organs. The same is true for anthrax. The cause of this requirement on the part of certain microorganisms, by virtue of which they can enter the body only through well-defined areas, is not entirely clear. It must rest on extremely fine biological differences in the cells and body juices as a result of which the bacteria are able to multiply only in particular areas or spread only from such regions. Some organs, indeed, may be able to destroy certain types of bacteria, thus preventing infection in them. It is possible also that some bacteria may depend upon the immediate presence of other varieties of organisms in order to bring on infection.

## FACTORS OF DISEASE PRODUCTION

Even after an infectious agent has penetrated the living tissue, the organism must multiply and be present in a certain quantity in order to produce disease. A prime characteristic of the pathogenic or parasitic bacteria is that after entrance into the living human or animal body they are able to increase up to the quantity necessary to bring about infection. The nonpathogenic saprophytes are lacking in this ability. Wyssokowitch[17] has shown that ordinary hay bacilli when introduced into the tissues do not multiply, but rapidly die out. When introduced in excess they may give rise to local inflammatory changes and a general febrile reaction, but all bacterial protoplasm, both of the parasites and saprophytes, has this capacity. The products of other saprophytes like the putrefactive bacteria can also give rise to disease symptoms if introduced in sufficiently large quantity (Panum[18]). The most striking property of the pathogenic infectious bacteria as contrasted with the saprophytes is this ability to multiply in the living body of man and animals.

The specific infectious agents, after their entrance into the body, do not necessarily multiply immediately and produce an infectious disease. They must first overcome the defensive mechanism of the body cells and juices which is put in operation upon the introduction of these agents. We speak of this defensive mechanism broadly as *natural resistance.* The outcome of the conflict between the bacteria and the antibacterial forces of the body depends upon a variety of conditions. On the one hand, we have the quantity of the micro-organisms actually introduced with the infectious material, their vitality or ability to develop, and their capacity to produce poisonous substances, *i. e.*, their virulence. On the other hand, we have the inherent protection afforded by the body structures and the body juices.

After the invading bacteria have developed in the tissues to the necessary numbers and have produced a specific injury to the body cells the infectious disease actually begins. A certain time always elapses between the moment at which the bacteria enter the body and the appearance of the symptoms of infection. We speak of this time as the *period of incubation*, the entire cycle of changes often being referred to as the *incubation* of the disease. The length of the incubation period depends upon the amount and the virulence of the infectious material, the place where it is introduced, and upon the protection against the bacteria afforded by the host.

The relationship between the *number* of bacteria and the length of the period of incubation can easily be determined experimentally. Thus in tetanus the dose can be so graded that a mouse injected with the organisms will show signs of infection on the fifth or sixth day, while if a larger dose is given the animal will show similar symptoms on the second day. Similar phenomena can be observed with a number of other infectious bacteria.

The *place* where the micro-organisms are introduced also has an important influence upon the incubation period in many infections, especially in those where particular organs must be attacked in order that symptoms of infection appear. For example, in rabies and in tetanus the infectious agents must be *fixed* to the central nervous system in order to produce disease. The nearer the portal of entry to the nervous system in any particular case, the earlier the first symptoms appear. Thus the incubation period is shorter in rabies with bites on the head than with bites on the lower extremities because the virus has a shorter path to travel in order to reach the specific organs. Similarly, Roux and Borrel[19] have shown that the incubation period in tetanus can be markedly shortened by the direct injection of the virus into the nervous system.

The incubation period can also be shortened by the direct injection of the bacteria into the blood channels or into the larger serous cavities. Here no protection against the development of the infectious micro-organisms is offered by the lymphatic system. In subcutaneous infections this protection of the lymphatics must first be overcome before the organisms get into the blood. Halban[20] has reported that after subcutaneous injection the pathogenic organisms are always found first in the regional lymphatic glands. Furthermore, direct injection into the blood naturally hastens the distribution of the bacteria and their penetration into distant organs.

In certain infections the length of the incubation period may be correlated with the severity of the disease. Rotter[21] has determined that all cases of tetanus in which the incubation period is less than ten days are very severe. This does not hold true for the majority of infections. In erysipelas following wounds Roger[22] has found that the severity of the infection does not apparently depend upon short incubation periods.

Finally, the length of the incubation period is not always the same in the same infection. Thus with very virulent streptococci symptoms may appear within twenty-four to forty-eight hours, while with streptococci of less virulence a number of days may elapse before the disease first begins to manifest itself. This holds true likewise for other infectious processes.

## SPREAD OF INFECTIOUS AGENTS IN THE BODY

After the incubation period is passed the *course of the infection* begins, caused by the activity of the micro-organisms within the body. We may divide this into *local* and *general* action, and must bear in mind the difference between the *mechanical* action of the organisms and the action of their poisons. With larger parasites, like such intestinal worms as *Bothriocephalus latus*, *Trichina*, and *Anchylostoma*, mechanical injury may play a certain rôle, but even here the poisonous action of secreted materials cannot be ruled out. Thus Tallqvist[23] has found that severe anemia may be produced by feeding substances containing *Bothriocephalus latus*. With certain bacteria like anthrax and hemorrhagic septicemia mechanical injury may also be important since thousands of organisms may be present in the blood and organs in infections with these micro-organisms and may act as foreign bodies. With the majority of bacterial infections like those from the tubercle bacillus, the diphtheria bacillus, the typhoid bacillus, and the cholera vibrio the mechanical effect on the tissues and upon the circulation cannot explain the severe general symptomatology; one must at once assume that poisonous substances are produced by the bacteria the absorption of which gives rise to the chain of symptoms observed in the infectious process.

This principle was postulated by Koch in Asiatic cholera, but Roux and Yersin[24] first demonstrated clearly in diphtheria the existence of specific bacterial poisons which can produce the same symptoms of disease as the living bacteria. Shortly after the diphtheria toxin was discovered by Roux and Yersin the tetanus toxin was found by Kitasato,[25] and since that time the action of bacteria has been generally traced to their production of specific poisonous substances. Thus far these specific toxins have been found in only a limited number of bacterial species. It must not be forgotten that the failure to obtain toxic substances from bacteria growing artificially in cultures is no proof that these bacteria do not elaborate toxic substances in the animal body where the conditions for their multiplication differ radically from those in the test-tube.

It has been pointed out above that the chief characteristic of the infectious or pathogenic bacteria, as distinguishing them from the non-pathogenic

saprophytes, is their ability to overcome the defensive mechanism of the body and multiply in the tissues and body juices. The mode of multiplication is not the same for all pathogenic bacteria and this is particularly brought out when we consider the *spread* of the infectious organisms. We may here differentiate four main types of infection (Wassermann and Keysser[26]):

A. The bacteria remain limited to the immediate neighborhood of their portal of entry. The best examples of this are tetanus, which regularly develops in the neighborhood of infected wounds, and diphtheria, where the infection is usually limited to the pharynx and adjacent structures. The evil effect of the multiplication of such localized organisms is to be attributed to the toxins which they elaborate. Other examples of localized infections are the so-called "Leichentubercles" due to the tubercle bacillus, furuncles due to staphylococci, and abscesses due to streptococci. With some of these organisms, such as the streptococci, localized growth is not a constant biological property, but results from the opposition to the development of the parasite excited by the tissues. Frequently, indeed, with bacteria which are able to bring about wide-spread infections localization indicates a milder and less dangerous disease. Furthermore, as a result of the localization of infections an immunity may arise which tends to prevent a general infection and permits only the local growth. Frequently the localization of the infection is not at the portal of entry, but at the neighboring lymphatic glands. With the tubercle bacillus we often have a localized infection of the bronchial lymphatic glands after the organisms have gained entrance through such portals of entry as the tonsils (Spengler,[27] Neumann[28]).

B. The bacteria show a progressive development and spread in the invaded bodies by direct extension. This is seen with such organisms as the influenza bacillus, which penetrates the epithelium of the respiratory tract and causes laryngotracheitis, bronchitis, and pneumonia. The cholera vibrios spread by continuity as typical epithelial parasites of the intestine. With streptococcus infections we may also see a spread by direct continuity as in the phlegmon of erysipelas and in streptococcus pneumonia. Pneumococci, tubercle bacilli, and leprosy bacilli likewise can spread by direct continuity.

C. The bacteria develop progressively in the tissues and spread by metastasis to new areas. Generally, distributed foci thus arise from the primary focus. When the infectious process is characterized by the appearance of fluid pus this condition is described as *pyemia;* this is especially apt to occur in staphylococcus and streptococcus infections. Other organisms, like tubercle, typhoid, leprosy, plague, glanders, and pneumococci, may also spread by metastasis. The bacteria travel by way of the lymph to the blood by means of which they are distributed to the organs where they produce the secondary foci. Frequently they stop and localize on the heart valves where infections arise from which emboli may be detached and pass to other parts of the body. A sudden metastatic spread of the tubercle bacillus results in the condition described as *acute miliary tuberculosis.* In chronic infections the metastases may form after considerable periods of time, as in leprosy, where the organisms may escape from the nodules of the disease into the blood and give rise to new nodules in distant areas. To the spread of parasitic agents by metastasis we must add those in which the bacteria are distributed by mechanical means and thus give rise to new infections. This mode of spread is seen in the distribution of organisms by the respiratory currents of air. Thus Nenninger[29] (under Flügge's direction) has shown that currents of air of slight intensity can carry bacteria from the mouth cavity to the lungs.

D. The bacteria develop primarily in the tissues and then spread by the blood to all the organs, multiplying in the blood itself. This is described as *septicemia*. It is seen in a variety of infections, such as those due to streptococci, plague, anthrax, and occasionally to staphylococci. Rarely *Bacillus typhosus* and *Bacillus coli* can give rise to true septicemias. In animals septicemias are especially due to species like swine-plague and fowl-cholera, which are often included in one group known as the hemorrhagic septicemia group. The multiplication of organisms in the blood and the production of a true septicemia is conditioned upon the disappearance of the normal bactericidal action of this fluid by means of which invading bacteria are destroyed.

In the earlier work of Lister and Pasteur the similarity between infection and putrefaction lead to the use of the term *sepsis* for all types of infectious processes with symptoms of general poisoning. At the present time the term *septicemia* is employed to indicate this condition. A *septic infection* thus means the spread and development of organisms in the blood. A combined septicemia and pyemia is called a *septicopyemia*. The occasional presence of bacteria in the blood in metastatic infections is not to be regarded as a septicemia, since here the blood merely acts as the vehicle of transport and the bacteria do not multiply in it. This condition is now usually described as *bacteremia*. It is frequently very difficult to draw the line between a true septicemia where there is an actual increase of organisms in the blood, and a bacteremia where organisms may be present in the blood in considerable numbers, as in lobar pneumonia, but do not multiply there.

While the methods by means of which pathogenic bacteria spread in the body may usually be determined for the various types of infection, it must be remembered that there are great variations and numerous combinations with nearly all the infectious organisms. The gonococcus may first localize on the uretheral mucosa, spread by contiguity to adjacent organs, travel by the blood, and form metastatic foci in various joints and on the cardiac valves. Similar conditions hold true for streptococci, staphylococci, and a number of other pathogenic bacteria. The reasons for this variation in the mode of spread are not entirely clear, but underlying factors must always be considered, such as the quantity and the virulence of the invading bacteria and the individual anatomical and biological factors which combine to make up what is called *disposition* or *predisposition*. Thus it may easily be shown experimentally that an anthrax bacillus of low virulence will produce a local abscess in a rabbit, while one of high virulence gives rise to a rapidly fatal septicemia. With infectious agents against which man or animals have a certain degree of resistance the *number* of the pathogenic bacteria has an important influence. Watson Cheyne[30] has estimated that with fowl-cholera organisms of moderate virulence, 10,000 to 300,000 bacteria injected subcutaneously in rabbits remain localized, while the injection of 3,000,000 leads to a general septicemia. With virulent organisms and sensitive animals the quantity of the infectious material plays no rôle. One anthrax bacillus or one short chain will produce a fatal infection in mice (Barber), or one short chain and a few hemorrhagic septicemia organisms a fatal infection in rabbits. The number of the pathogenic parasites in general must not go below certain limits in order that infection follow their introduction; this number probably varies greatly with the type of organism. The necessary number also varies with the portal of entry. Findel[31] and Laffert have shown that 200 to 300 tubercle bacilli can produce severe lung

tuberculosis in guinea-pigs, while it requires several million bacilli to produce infection from the intestines, and even with large quantities the results are by no means certain. In general, man may be regarded as a sensitive species for some infections and a resistant species for others, under natural conditions probably only a few organisms getting into the body. How much of a rôle the number of bacteria plays in human infections is still open to discussion and, naturally, accurate observations upon this point are lacking.

The *resistance* of the body to the growth of bacteria has the greatest influence upon their spread. The same quantity of anthrax bacilli which will produce a fatal septicemia in mice and guinea-pigs will give rise to a local inflammation only in the more resistant rat. The same principle holds true for a variety of other infections.

The spread of infectious organisms is dependent also upon various *mechanical factors*, such as aspiration, the breaking down of localized foci of infection and the passage of bacteria into the blood or lymph, the solution and detachment of cardiac vegetations, etc. Cases vary greatly, especially in human infections, and it is sometimes very difficult or impossible to determine the path by which the organisms have spread from one part of the body to another.

In general, bacteria are now recognized to spread more widely than was formerly believed and we now know that many infectious agents appear temporarily in the blood during the progress of an infection. Petruschky[32] has observed streptococci in the blood in streptococcus infections and in erysipelas, and yet these cases may go on to complete recovery. In croupous pneumonia pneumococci may be present in the blood in 25 to 35 per cent. of the patients (Banti, Kohn). Conradi[33] has found typhoid bacilli in the blood in 50 per cent. of the cases and Kayser has found them in 98 per cent. The presence of pathogenic bacteria in the blood cannot therefore be regarded as such a bad prognostic sign as was formerly supposed. Much depends upon the number which may be isolated and the length of time during which they are present.

In the *localization* of the bacteria in the body of man the majority show some preferance for certain organs and tissues. Leprosy bacilli tend to invade and grow in the peripheral venous system, cholera vibrios in the epithelium of the intestinal mucosa, typhoid bacilli in the lymphoid follicles of the intestine and in the mesenteric glands, and dysentery bacilli in the mucosa of the colon. The same principle holds true for the pyogenic organisms. The gonococcus and the staphylococcus tend to localize in the joints, the pneumococcus in the lungs. This predilection for certain organs or tissues is seen especially with the so-called filtrable viruses. The virus of hog-cholera, after intravenous or subcutaneous injection, always produces the typical intestinal ulceration, while the virus of poliomyelitis localizes in the gray substance of the spinal cord. Toxins also have a special affinity for certain tissues. The tetanus toxin has an affinity for the nervous system and the dysentery toxin for the intestinal mucosa. Flexner and Sweet[34] believe that the latter is secreted by this mucosa.

The reason for the localization of bacteria and their products in particular organs and tissues is not entirely clear. In infections from pathogenic organisms which tend to spread widely, like the streptococci, the localization in special areas is probably due to the resistance offered by the defensive mechanism of the body, and such localization is usually a favorable prognostic sign.

Biological differences have also been noted on the part of the host which tend to modify the pathogenicity and invasive power of bacteria. In times

when Asiatic cholera is epidemic the cholera vibrio may be found in the dejecta of normal individuals who have no symptoms of the disease. In communities with badly contaminated water supplies, after an epidemic of typhoid fever has once been started, probably a large number of people swallow the typhoid bacillus, and yet only a certain proportion of the population develops enteric fever. The *age* of the individual has a marked influence in determining the type of disease from certain micro-organisms. *Bacillus pyocyaneus* never invades the body from superficial wounds in adults, whereas such invasion is common in very young children (Schimmelbusch,[35] Kossel[36]). Some organs can be infected in one age period, but not in another. The gonococcus can invade the buccal mucosa in the newborn (Rosinski[37]), but such an invasion has never been observed with certainty in adults.

Finally, *physical* and *chemical properties* of the tissues at the portal of entry may play a rôle in determining infection. Dead necrotic tissue favors the development of pyogenic organisms, the tetanus bacillus, and other anaërobes, possibly by furnishing nutritive materials for the growth of the bacteria. This principle was demonstrated experimentally by Dorst,[38] who found that after the production of a hematoma in rabbits, the injection of 1/100 c.c. of a pneumococcus culture was followed by a fatal infection, while 1 c.c. of the same organism was required as a lethal dose for a normal control.

The character of the tissue invaded may be of moment in establishing the type of infection. The streptococcus finds some opposition to its spread in the hard connective tissue of the finger and produces only a localized inflammation (a panaritium), while it grows readily in loose connective tissue and may cause an extensive phlegmon.

Several suggestions have been offered to explain the mechanism by means of which the infectious organisms are able to invade the organs and tissues, *i. e.*, their pathogenicity. According to some authors (Roger,[39] Kruse[40]) the bacteria give off materials which neutralize the normal protective substances of the body and predispose it for invasion. Kruse has called these materials *lysins* and believes that the capacity to grow and invade depends on the quantity and kinds of lysins furnished by the bacteria. Van de Velde, however, has found that with staphylococci lysins were produced by virulent and non-virulent strains in the same amount, and pathogenicity in this species therefore cannot be correlated with such products. It is possible that pathogenicity depends on the resistance offered by the bacteria themselves to the protective substances of the body. Danysz[41] has shown that the infectiousness of the anthrax bacillus is increased after passage through susceptible animals. At the same time the organism is found to be surrounded by viscid capsules which apparently serve to protect it from the body juices. This is evidently an exceptional mechanism and can only explain the pathogenicity of such encapsulated organisms as the anthrax bacillus, the Friedländer bacillus of pneumonia, or the "gas bacillus" of Welch and Nuttall. In the long run we must assume that all pathogenic bacteria secrete some sort of material which injures the cells in their immediate vicinity and that these injured cells by virtue of their loss in protective substances permit the development of these same micro-organisms in them. Under normal circumstances the protective mechanism of the body suffices to destroy the majority of the bacteria which are introduced. A few species are endowed with the capacity of producing materials which overcome the protective mechanism of the body, possibly toxins, possibly aggressins or endotoxins. As a result the pathogenic bacteria begin to increase and continue to multiply, constantly overcoming the defensive body mechanism. The method by means of which bacteria invade must eventually be explained

by *mechanical factors*, lymph and blood-stream, stream of body juices, permeability of cells, etc., provided always that certain species are especially able to overcome certain body juices as the streptococci overcome the blood. It must be remembered that of the thousands of organisms in the world only a small number are really pathogenic.

Bail[42] believes that the invasive properties of bacteria are explained somewhat by the so-called *aggressins*. Bail has shown that in guinea-pigs injected with several multiples of a fatal dose of bacteria (*Bacillus anthracis*) the fresh peritoneal exudate freed from cellular elements has remarkable properties. When injected with a *sublethal* dose of the same organism it produces an acute fatal infection; when injected with a fatal dose or several multiples it produces a more severe infection than from the bacteria alone. Finally, if the animals be injected with a mixture of bacteria, protective serum, and aggressins, the protective serum has no action and the animals die. By repeated injection the animals can be actively immunized. This immunity is neither bacteriolytic or antitoxic, but *anti-aggressive*, and is characterized by a powerful phagocytosis and a suppression of bacterial multiplication. Thus when bacteria are introduced into the body certain materials (aggressins) are produced which favor the development of bacteria and combine with or injure the protective substances of the body. Wassermann and Citron regard the natural aggressins of Bail as extractive substances of the bacterial bodies which can be obtained from aqueous extracts of the cells. The aggressins are regarded as endotoxins by Doerr[43] and by Sauerbeck,[44] liberated by the death of the organism in the tissues.

## VIRULENCE OF THE BACTERIA

By the virulence of bacteria is meant the strength of their pathogenic activity in the sensitive or non-resistant body or the sum of their disease-producing properties. Many authors define virulence as infectiousness or the ability to multiply in the body, and differentiate between it and toxicity, or the ability to produce toxins. Infection and intoxication are so closely bound together in the living individual that it is hard to differentiate between the extension of the bacteria and the action of non-viable materials. With species like the diphtheria bacillus and the tetanus bacillus the infectiousness is much less than the toxicity. Here the grade of virulence is determined by the amount of toxin production. With species like the tubercle bacillus the lesions depend on the presence of the organisms in the tissues, and here virulence is related to the ability of the bacteria to multiply and spread. The *cause* of virulence in bacteria is not clear. In some instances it goes hand in hand with the ability of the organisms to bind the immune body or amboceptor, as has been shown for the cholera vibrio by Pfeiffer and Friedberger[45] and by Wassermann and Strong.[46] This is not a general law and does not hold true for other organisms like *Bacillus typhosus*. Bail identifies virulence with the ability to elaborate aggressins and to overcome the protective mechanism of the body.

The *grade* of virulence varies greatly in the species of pathogenic bacteria and in the strains of the same species. It can be raised and lowered by a number of procedures and frequently is lowered for one species of animal and remains the same or increases for another. A high virulence of certain organisms for one species does not mean a high virulence for all animals. That is to say, there are *qualitative* as well as *quantitative* differences in the virulence of pathogenic bacteria for different species. This was originally pointed out by Pasteur[47] in swine-erysipelas and by Knorr[48] for the streptococcus. The virulence of the streptococcus may be increased for mice and

at the same time lessened for rabbits, and *vice versa*. The virulence of organisms for such species is thus conditioned upon the ability to break down the protective mechanism which that species exerts against the particular bacteria.

The virulence of bacteria also depends closely upon the ability of the parasites to adapt themselves to unfavorable conditions in the host, just as certain protozoa, like the trypanosomes, become resistant to trypanocidal substances and to immune bodies in the infected animal. Jensen[49] has found serum-fast strains of an organism from calf dysentery, Wassermann and Ostertag[50] serum-fast bacilli from swine-erysipelas, and Jaffé and Besserer serum-fast typhoid bacilli. In the adaptation of organisms to the host many individual strains must arise which have varying ability to overcome the defensive mechanism of the body. Those which overcome it completely are virulent, while those which fail to overcome it are non-virulent. Whether morphological and cultural differences also arise in this adaptation has not been settled, but in general no satisfactory evidence of such morphological variation has thus far been brought, except possibly in capsule formation in the anthrax bacillus.

**Methods of Changing Virulence.**—The virulence of bacteria is subject to great variation and increases or decreases under a number of circumstances. The chief occasions in which loss of virulence is seen are the following:

1. *Artificial Cultivation on Media outside the Animal Body.*—The majority of bacteria show the highest degree of virulence on transfer from the body of infected animals to other animals without intervening culture. For example, blood containing the anthrax bacillus is more virulent, organism for organism, than cultures of this bacillus. We do not know why this loss of virulence takes place in cultures. Injurious products like *acids* or *alkalies* may arise in the culture-media which injure the organism. Thus the pneumococcus is extremely sensitive to an excess of acids, as well as the streptococcus and the diphtheria bacillus. Both streptococci and diphtheria bacilli lose virulence more quickly in media containing glucose or glycerin, from which acids are produced by the growth of the organisms, than in media free from these ingredients. Bacteria may also produce strongly alkaline substances which reduce the virulence, as in the case of the cholera vibrio. In some instances *ferments* apparently develop which injure the living bacteria, like the pyocyanase of *Bacillus pyocyaneus*. The loss of virulence may be limited to one generation, the virulence being restored on transfer to fresh media. At other times the virulence is lost completely and the organisms are permanently altered.

2. *Subjecting Organisms to High Temperatures.*—The action of heat in diminishing virulence was first demonstrated with *Bacillus anthracis* by Toussaint,[51] who heated blood containing this organism to 55° C. for ten minutes and found that it was less virulent than the original blood. It was subsequently shown by Pasteur, Chamberland, and Roux[52] that the virulence of the cultivated organism could be lowered by careful heating and that this property was retained on transfer to new media. Thus *new strains* (vaccines) were produced. By cultivation of *Bacillus anthracis* at 42° to 43° C. in broth for a week the bacilli became non-virulent. All grades of virulence could be obtained by varying the length of time during which the organisms were kept at this temperature and the new virulence was retained for subsequent generations on artificial media. High temperatures reduce the virulence of other organisms also, like the diphtheria bacillus, the typhoid bacillus, and the cholera vibrio.

3. *Subjecting Organisms to Sunlight.*—It has been reported by Arloing[53] that the virulence of *Bacillus anthracis* may be diminished by exposure to the action of the sun's rays. This may be due to the destruction of some of the bacteria by the sunlight, but Santori[54] has found that the organisms are really weakened in virulence before they are killed.

4. *Drying* has often been employed to diminish the virulence of organisms. The virus of rabies becomes less virulent by drying over alkali, as was originally shown by Pasteur. In this procedure it is not entirely clear whether the loss in virulence is due to the destruction of some of the infectious agents in the material or to an actual change of pathogenicity in this agent. According to Höyges the loss of virulence in rabies virus from drying is to be attributed to destruction of some of the organisms.

5. *The Addition of Chemicals to the Culture-media.*—It has been proved by Chamberland and Roux[55] that the anthrax bacillus cultivated in broth containing 1/800 to 1/600 volume of carbolic acid or 1/600 to 1/500 volume of potassium bichromate becomes non-virulent after ten days. The trichlorid of iodin has been used for the same purpose on diphtheria and tetanus by von Behring and Kitasato,[56] Lugol's solution by Roux and Martin,[57] while carbon bisulphid has been employed by Ehrlich for the tetanus bacillus. With these species the loss in virulence is probably due to the destruction of the toxins.

6. *Passage through Animals.*—Certain organisms lose in virulence by passage through certain species of animals. It has been shown by Pasteur that swine-erysipelas loses its virulence by passage through rabbits, while the virus of rabies shows the same change by passage through apes. Streptococci become less virulent for mice by passage through rabbits. In vaccinia it is believed by Fischer[58] that the vaccine virus is true variola virus lessened in virulence by passage through the calf. An adequate explanation for loss in virulence by passing infectious agents through animals has never been presented.

In consequence of the many factors whose action occasions a loss in virulence, it is now recognized that virulence can best be retained by the use of media containing body juices, blood-serum, exudates, etc., by protecting organisms from sunlight and drying, by keeping them at a low temperature, and by employing chemicals to neutralize the acids or alkalies which develop in culture-media.

The virulence of bacteria can be increased by several procedures, of which the following are the most important:

1. *Frequent Transfer.*—Bacteria have their virulence increased by transfer from one medium to another, on media especially appropriate for the species. This suffices for all those varieties in which virulence depends on numbers and toxins. For organisms which are injured by acids Martin[59] recommends a medium made up of self-digested pig's stomach and equal parts of calf meat broth. On this medium the diphtheria bacillus develops without producing acid. For the pneumonococcus Hiss has suggested the addition of powdered marble (*calcium carbonate*) to the medium by means of which the acids are neutralized.

2. *Repeated Passage of Organisms through Sensitive Animals.*—An increase of virulence by this method was first obtained by Davaine for the bacillus of mouse septicemia. Later Pasteur increased the virulence of swine-erysipelas by the use of pigeons, reducing it by using rabbits. In general, the passage of organisms through sensitive animals increases the virulence for practically all animals, but occasional exceptions to this rule are found.

The rat bacillus of Danysz loses its virulence for other animals on passage through the sensitive rat. In applying this method it is advisable to let the animals die and obtain cultures from the organs. Kolle and Martin have shown that in bubonic plague organisms from the lungs are especially virulent. It is possible that the organs themselves may have some effect on the bacteria growing in them.

Both naturally sensitive animals and animals made sensitive by certain procedures, such as cutting the spinal cord, injection of aggressins, etc., can be used for this purpose. In some instances the virulence can be so increased that animals naturally refractory are sensitive to the organisms with enhanced virulence. This has been found by Sawtschenko[60] for the anthrax bacillus in pigeons with the spinal cord severed. After passage through such animals the strain of anthrax becomes virulent for the naturally resistant pigeon. The same phenomenon has been observed by Fermi and Salsano[61] for avian tuberculosis and guinea-pigs, the resistance of which was lowered by the injection of glucose and lactic acid. According to Metchnikoff[62] and Bordet[63] the passage of organisms through an immune animal increases virulence more than the passage through the normal animal. Here a possible explanation is that the less virulent cells in the strain die out, leaving behind the highly virulent.

3. *The Addition of Chemicals to the Organism.*—Arloing and Cornevin[64] have enhanced the virulence of the anthrax bacillus by the addition of 2 per cent. lactic acid and glucose. Potassium nitrate, sodium phosphate, and inorganic salts enhance the virulence of the cholera vibrio (Blachstein[65]). Roux believes that the presence of oxygen increases the virulence of the diphtheria bacillus, and Hueppe[66] has found that that of the cholera vibrio is increased in the absence of oxygen. Brieger and Cohn[67] have shown that the virulence of the tetanus bacillus is strengthened by extracts of putrefying flesh. Occasionally the virulence of bacteria seems to increase on cultivation in media containing certain organs, like the liver and kidney, or with special kinds of peptone, etc.

**Virulence of Bacteria for Man.**—We have no method of judging of the virulence of organisms for man, and our estimation of the strength of any particular species for man rests upon the determination of its strength for animals. This may be fallacious, since strains which are highly virulent for animals are not necessarily so for human beings. Koch and Petruschky[68] have reported that streptococci with maximum virulence for rabbits will not cause erysipelas in man. Nevertheless there is a certain general correspondence. Vagedos has indicated that tubercle bacilli from severe cases in man are very virulent for rabbits and rats. Stuertz believes that the virulence of pneumococci for mice corresponds to its virulence for human beings, and in general pneumococci from severe or fatal cases of pneumonia are highly virulent for both mice and rabbits. Frequently highly virulent streptococci from human sources prove to be highly virulent for animals.

## SIGNS OF INFECTION

**Local Reactions.**—The action of an infectious agent is *local* and *general*. By local action we mean the changes which pathogenic bacteria or their poisons bring about in the tissues in which they happen to be present. These changes may be *specific* for certain species of micro-organisms, or be *generally inflammatory* in character with the production of pus or proliferative tissue, resulting in nodules or tumor-like masses (granulation tumors of Virchow or infectious granulation tumors of Ziegler). All grades of inflammation arise from the action of pathogenic bacteria or their poisons on tissues,

serous, fibrinous, purulent, membranous, hemorrhagic, necrotizing, gangrenous, and proliferative with nodule production. Frequently combinations of these changes occur. There is no regular type of inflammation with any pathogenic bacteria. Certain local reactions are often associated with certain species of micro-organisms, such as tubercles with the tubercle bacillus and glanders nodules with the glanders bacillus, but both the tubercle bacillus and the glanders bacillus can produce true inflammation with purulent exudate.

The regularity with which inflammation is associated with the growth of pathogenic bacteria in the tissues is so marked that for a long time it was doubted whether inflammation ever occurred without bacteria. It has now been proved, however, that true inflammation follows the introduction of sterile irritating materials, like mustard or croton oil, beneath the skin, and also trauma.

The majority of infectious bacteria produce local changes at the portal of entry. There are, however, certain exceptions to this rule. Streptococci may get into the tissues and spread rapidly in the body without a local reaction at the site of entrance. In bubonic plague the organisms enter the body through the skin without local lesions, producing lesions first in the neighboring lymphatic glands. Infections of this character without reaction at the portal of entry are called *cryptogenetic*. It must be remembered with all such infections that the portal of entry is often difficult to find and that in many instances the local lesion may be insignificant in character and heal rapidly. Nevertheless true cryptogenetic infections seem to occur. This question has been much debated in regard to the tubercle bacillus. Cornet[69] believes that this organism may pass the uninjured mucosa without leaving lesions in its wake. Baumgarten[70] and Tangl[71] maintain that local lesions always occur.

Infectious micro-organisms frequently spread rapidly from the portal of entry. Schimmelbusch[72] has shown that when *Bacillus anthracis* is inoculated on fresh bleeding wounds in mice it may be found in half an hour in the lungs, liver, spleen, and kidney. Similarly *Bacillus pyocyaneus* may be found in five minutes. According to Neisser *Spirochæta pallida* spreads rapidly in lues from the local lesions in apes.

Other pathogenic agents, like the virus of rabies, remain much longer at the portal of entry before spreading. Bombicci[73] has found that after the injection of rabies virus into the anterior chamber of the eye in rabbits the development of the disease may be prevented by the enucleation of the eye the following day.

The question is of great practical importance, since it is sometimes necessary to determine whether the removal of the primary lesions in infections will have any beneficial effect upon the course of the disease.

The local changes produced by micro-organisms are not to be regarded as due to their presence as mechanical agents, but to the biological reaction between the living organisms or their products and the tissue cells. Nor is it always necessary that the organisms be living. It has been proved by Prudden and Hodenpyl[74] that the injection of dead tubercle bacilli into the circulation will produce tubercles in the tissues. Local changes can easily be produced by the germ-free diphtheria toxin, these lesions corresponding closely to the lesions found in the natural disease. Very early in bacteriology Pasteur pointed out that pus could be produced by the injection of dead pus cocci.

The mechanism of the production of *purulent inflammation* has been especially investigated and there are many intricate changes in the phenom-

enon. It has been found by Pfeiffer[75] that bacteria possess the power of attracting leukocytes, this power being known as *chemotaxis*. Subsequent investigations have revealed that nearly all bacteria possess positive chemotactic materials which attract leukocytes and produce pus. According to Roemer[76] extracts can be obtained by boiling and macerating bacteria which act as pus-producing inflammatory substances, and these substances give protein reactions. This inflammatory reaction due to the bacterial protoplasm must not be confounded with the local specific action of bacterial toxins. Probably all bacteria can produce local changes by means of their bacterial protoplasm, even the harmless saprophytes.

The local changes produced by bacteria vary greatly with the type of organism, its virulence, and with the animal which is infected. The Friedländer bacillus causes a purulent exudate in the pleural cavity, while the typhoid bacillus produces sometimes a serous exudate, sometimes a purulent, and rarely a hemorrhagic. The streptococcus usually produces a purulent inflammation in the same region, the pneumococcus, a serous exudate. In general, organisms of low virulence tend to cause purulent exudates, but the same organism can give rise to the most varied type of inflammation, depending on the anatomical and biological properties of the tissues at the site of infection.

Man is generally more disposed to purulent inflammation than animals; minimal quantities of bacteria and their products, which are often borne by animals without any reaction, may produce strong inflammatory changes in human beings.

Species of animals also differ greatly in the type of the local lesions which follow the invasion of bacteria. Thus *Bacillus anthracis* causes a local serous exudate in mice, a purulent inflammation in rats.

Finally, the body tissues may have a definite disposition for certain types of inflammation. Schrank has observed that serous exudates are apt to occur in infections of the periosteum of the bones, while suppurative inflammations develop in the bone-marrow. Herman has found experimentally in rabbits that different parts of the body are differently disposed to infection. The most severe suppurative inflammation occurs in the anterior chamber of the eye, the mildest in the peritoneal cavity.

Metchnikoff and his followers regard inflammation as a *protective mechanism* which the body exerts toward the infectious agent, a struggle between the organism and the tissues. Positive chemotaxis thus plays an important rôle, since phagocytes are brought into the affected area and engulf the organisms. Buchner, Wassermann, and others claim that the extravasated serum at the site of inflammation also tends to destroy the bacteria. In all probability both the serum and the leukocytes are needed in order that the invading bacteria be disposed of.

To some extent inflammation may be a healing process, and there is no doubt that the local destruction of organisms by serum and phagocytes assists markedly in limiting infections. Wassermann and Keysser believe that the powerful local reaction is not the cause of this limitation, but is produced by the general or local resistance or immunity. Guinea-pigs partially protected with diphtheria antitoxin show a local reaction with necrosis after subcutaneous injection with diphtheria toxin, while normal guinea-pigs die rapidly from general intoxication without local reaction. Man may show a local inflammation from *Bacillus typhosus* when protective substances are circulating in the blood-stream. In such cases the local reaction may not be the *cause* of the localization of the inflammation in

diphtheria and typhoid, but rather the *result* of the general immunity or an expression of it.

In general, we may regard local reactions as a possible cause of the limitations of infections and their change from a severe to a mild course, as the result of general natural or artificial resistance, or as the result of the injurious action of the organism and its products on the tissues. With our present knowledge we have no right to regard the local lesion as a teleological process.

**General Reactions.**—In the course of the distribution of infectious agents and their products in the body certain changes take place in the blood and organs, and both toxic materials and protective substances may at times be demonstrated in them. Brieger[77] has found tetanus toxin in the blood of an amputated arm in a case of tetanus and Nissen[78] has demonstrated tetanus toxin in the circulating blood in a severe case of this disease. Tetanus toxin has been shown by Kitasato[79] in the organs of animals with tetanus, in those of adults and in the organs of infants in tetanus neonatorum. Diphtheria toxin may be present in the organs and blood of animals in diphtheria infections, and occasionally in man, but only in severe cases. Uffenheimer[80] has obtained it in one instance from the circulating blood during life. Diphtheria toxin has been observed in the urine by Roux and Yersin and tetanus toxin in the urine by Brieger and Wassermann.

As a result of the presence of bacteria and their poisons in the body and of the action of its protective mechanism, various substances are produced which circulate freely in the blood-stream and may be found in practically all the organs and tissues. These substances are *antitoxins*, *bactereolysins*, *complement-fixing bodies*, *precipitinins*, and *agglutinins*. At the same time the body may become hypersensitive to new infections with the same type of material and other substances may be demonstrated, as in the reaction known as *anaphylaxis*.

**Fever.**—The most frequent sign of infection is an elevation of body temperature known as fever. All types of infection can cause this elevation, but fever is not a necessary accompaniment of infection. Thus tuberculosis may exist for long periods without fever. Small localized staphylococcus infections also may run along without fever which develops, however, in wide-spread infections with this organism. This elevation of temperature is related to the presence of the infectious material in the body. In man a small quantity of dead bacteria injected subcutaneously causes fever, as in typhoid vaccination. Various bacterial products give rise to fever in animals. Charrin and Ruffer have shown that *pyocyaneus* toxin produces fever in rabbits.

The mechanism of fever production is still somewhat obscure. Certain authors believe that a special fever-producing substance known as a *pyrotoxin* is elaborated by bacteria. Practically all organisms have pyrogenic products which raise the temperature in small doses and lower it in large doses. This has been shown for *Bacillus coli* by Roger, for *Bacillus typhosus* by Sanarelli, and for dead hog-cholera bacilli by Metchnikoff. Buchner's bacterial proteins likewise have a pyrogenic action. According to Centanni[81] fluid cultures of both saprophytic and pathogenic bacteria contain a pyrogenic substance soluble in water, but not in alcohol. This has been called *pyrotoxin*, but final proof that it is the cause of fever has not been brought.

In accordance with modern investigations fever should probably be regarded as a kind of protective mechanism of the body against materials which are foreign or noxious to it, and which is intimately associated with changes in metabolism. In some infections fever is to be regarded as a favorable sign.

**Leukocytosis.**—Infections are always accompanied by changes in the white cells of the circulating blood. These were first studied by Roemer with Buchner's proteins and by Kanthack[82] experimentally in animals injected with bacterial products. In the typical reaction a *hypoleukocytosis* or *leukopenia*, a diminution in the number of leukocytes, first occurs, and is followed by a *hyperleukocytosis*, an increase in number, which reaches its highest point about nine hours after the infection starts and lasts forty-eight to seventy-two hours. In men we seldom have an original leukopenia, but in animals this is common in experimental infections; it is probably due to a withdrawal of the white cells from the circulation and not to their destruction. It may occur in man in uncomplicated typhoid fever, in measles, and in other infectious processes where the absolute number of leukocytes in the blood is lessened at the cost of the polymorphonuclear neutrophiles.

Hyperleukocytosis may be regarded as the result of *positive chemotaxis*, bacteria and their poisons drawing the leukocytes from the hematopoietic system into the blood. Similarly, *hypoleukocytosis* may at times be regarded as the result of a *negative chemotaxis*. Löwit[83] has shown experimentally that in leukopenia the polymorphonuclears are not always destroyed, but are present in greater numbers in the central capillaries, as in those of the lung, being driven out of the blood-stream. In bacterial infections the leukocytosis is generally due to an increase of the polymorphonuclear neutrophiles. In infections with animal parasites an increase of eosinophiles or an eosinophilia usually occurs, as was first shown by Brown[84] for trichina infections.

Leukocytosis like fever is in general an expression of the action of the defensive mechanism of the body against invading bacteria. The presence of leukocytosis is frequently a good prognostic sign. Its absence may be a bad sign as in severe and fatal cases of pneumonia.

Other signs of infection are enlargements of the lymphatic glands, acute splenic tumor, anemia from the destruction of the red blood-cells as in severe streptococcus disease, and occasionally a tendency for the formation of hemorrhages, such as Howard[85] has noted in *Bacillus mucosus capsulatus* invasions of the body. At autopsy characteristic lesions are usually present such as enlargement of the spleen and lymphatic glands, parenchymatous degeneration of the cells of various organs and amyloid degenerative changes in the tissues of the nervous system.

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## Chapter II

## MIXED INFECTIONS

It was earlier believed by some authors that the association of two or more organisms was necessary in infections and this association was spoken of as *symbiosis*. At the present time there is not a single infectious agent in which certain proof has been brought that the agent cannot produce infection by itself. Other bacteria may play a rôle in preparing a way for the infection as occurs with tetanus which invades tissues already injured by necrotizing bacteria. The tetanus toxin can have the same action since washed tetanus spores do not cause infection (Vaillard and Rouget[1]) and an aseptic hematoma may be invaded by the tetanus bacillus. We frequently find two or more species of organisms together, especially in such body cavities as the lungs which communicate directly with the air. Here it may be very difficult to determine whether all the species are active or not, and frequently one species is pathogenic and the other saprophytic. In the blood and in the internal organs two or more types of bacteria are not uncommonly found in infections. We speak of this association of organisms as a *mixed infection*.

In other instances we have *secondary infections* in which the *primary* agent is followed by a *secondary invader*. This occurs frequently in smallpox where the streptococcus invades the tissues already changed by the smallpox virus. Sometimes in mixed and secondary infections both organisms are admitted to the body at the same portal of entry. This is seen in diphtheria in which a secondary invasion by throat streptococci occurs. At other times the primary agent may make a portal of entry for the secondary, as in typhoid fever or Asiatic cholera, where the mucous membrane of the intestine is injured and secondary invaders enter.

Several efforts have been made to show that one infection may have a favorable influence on another. Emmerich and Di Mattei[2] injected rabbits with streptococci and found them later resistant to the anthrax bacillus. Pawlowsky[3] showed that organisms like the Friedländer bacillus, *Bacillus prodigiosus*, and staphylococci, when injected into animals, seemed to render them more resistant to invasion by *Bacillus anthracis*. Here the beneficial effect is not necessarily due to the first infection, since we now know that a number of substances like sterile broth, urine, and normal serum are capable of increasing the resistance of animals. In combined anthrax and *Bacillus pyocyaneus* infections, where the infection with *Bacillus anthracis* seems to be favorably influenced by the other organism, the reaction may be due to the *pyocyanase* described by Emmerich and Löw.[4] This is secreted by *Bacillus pyocyaneus* and has the capacity of dissolving organisms like the anthrax bacillus. At the present time we may say that a favorable influence of one infection on another is not proved, while the unfavorable influence is everywhere admitted. We know also that the virulence of organisms may be increased by bacterial association. It has been demonstrated by Monti[5] that killed cultures of *Proteus vulgaris* enhance the virulence of pneumococci, streptococci, and staphylococci, while Fessler[6] has shown that living proteus cultures do the same thing for streptococci. The virulence of the pneumococcus is raised by a simultaneous injection of anthrax (Pane[7]). According

to Roux and Yersin[8] the administration of streptococci and avirulent diphtheria bacilli leads to fatal diphtheria in guinea-pigs, but van Dungern[9] believes that here the virulence of the streptococci not of the diphtheria bacilli is raised. In all such experiments we must remember that the fatal result may be due to the direct summation of the two infections. Naturally the body is less able to bear two or more diseases than it is to bear one. Probably the increase of virulence even if it occurs is not the important thing. Rather must we believe that the injury produced by the primary agent so uses up the resistance that the secondary agent grows in easily. With the advent of the second organism a new disease picture is established which may be quite different from the original disease. We may have a septic condition from the invasion of the blood by streptococci in typhoid fever and the so-called "hectic fever" in tuberculosis may be due to secondary infection. With the so-called filtrable viruses secondary infections are the rule. In hog-cholera, a disease due to a filtrable virus, we nearly always have a secondary invasion by the hog-cholera bacillus.

Various types of association of pathogenic bacteria may be seen in mixed infections. Wassermann and Keysser[10] classify them as follows:

1. Association in which the different kinds of organisms remain localized, as in the so-called "Leichentubercle" where both pyogenic cocci and the tubercle bacillus are present, and in the anaërobic infections of wounds where *Bacillus Welchii* is frequently found associated with *Bacillus sporogenes*.

2. Association in which both the primary and secondary species spread in the body. This is seen in mixed infections from the typhoid bacillus and streptococci, the typhoid bacillus and *Bacillus coli*, the plague bacillus and streptococci, etc. Infection with both typhoid and paratyphoid bacilli has been reported by Conradi[11] and Kayser,[12] and Beckers[13] has cultivated both these species from the blood in such a combined infection.

3. Association in which the primary infectious agent remains localized while the secondary spreads rapidly. This is seen especially in the exanthems like smallpox and measles, where a secondary invasion with the streptococcus converts a mild affection to a severe and fatal disease. Complications and metastases may be caused by the secondary invaders, as in the purulent inflammations in typhoid fever and bubonic plague. Here frequently both the primary and the secondary infectious agents may be found in the lesions, *Bacillus typhosus* with pyogenic cocci, *Bacillus pestis* with pyogenic cocci.

4. Association in which the primary agent spreads while the secondary agent remains localized. This is seen especially in lesions exposed to the air. In tuberculous cavities in the lungs various bacteria may develop in the necrotic tissue, while the tubercle bacillus continues to attack fresh tissue. In typhoid fever intestinal bacteria like *Bacillus coli* may invade the tissues already the seat of disease, while the typhoid bacillus gets into the blood and causes a fatal septicemia. In bubonic plague Kashkadomoff[14] has shown experimentally in mice and guinea-pigs that in combined infections with *Bacillus pestis* and staphylococci, the staphylococcus infection may remain localized and the animals die of plague septicemia.

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## Chapter III

## INFECTION BEFORE BIRTH

Infection of the fetus before birth is possible from a number of causes. The sperm of the father or the ovum of the mother may be infected at the time of conception, in which case bacteria may multiply in the developing embryo. This is known as *germinal infection*. Or the fetus may be invaded by bacteria through the placental circulation. This is called *placental transmission* or *placental infection*. There is, of course, no real inheritance of infection according to modern views, since all inheritance lies in the chromatin of the germ plasm. What we may have is infection of the sperm, ovum, or placenta. Infections of this character are admittedly extremely rare and practically negligible compared with contagion during life.

**Placental transmission** has been studied experimentally by a number of investigators, Baumgarten[1] and Wolff[2] among others. On the basis of his observations with anthrax on pregnant rabbits and guinea-pigs Wolff concluded that the normal placenta acts as an efficient barrier to prevent the passage of bacteria. This holds true also for vaccinia in man since children of women vaccinated during pregnancy are sensitive to vaccine virus. Injury in the placenta, especially minute hemorrhages, may allow the passage of organisms. According to other authors, Eberth,[3] Ernest,[4] and Hildebrandt, who worked with typhosus infections, gross lesions of the placenta must be present in order to permit organisms to pass from mother to fetus. Lesions of the placenta are found in anthrax infections in guinea-pigs and the anthrax bacilli pass to the fetus, while in rabbits no placental lesions occur and no transmission takes place. Other authors believe that placental transmission is possible without lesions of this organ. Birch-Hirschfeld[5] claims that the anthrax bacillus can grow from the blood spaces of the maternal placenta into the tissue of the fetal placenta, while Lubarsch[6] holds that pathogenic bacteria can pass through the epithelial and capillary spaces.

Much depends on the anatomical structure of the placenta itself. Usually the normal placenta of rabbits and goats permits transmission, while the mouse placenta does not. The human placenta resembles the rabbit's placenta and is permeable for bacteria.

Other factors in placental transmission are the severity of the disease in the mother, the virulence of the organisms concerned, and the length of the infection. Transmission is possible when the bacteria are present in large numbers in the intravillous spaces. The frequency of infection varies with the species of organism. The anthrax bacillus appears late in the placenta in anthrax infections, shortly before the death of the animal, and hence transmission is rare. With the pneumococcus and with the pyogenic cocci the bacteria appear early in the placenta. On this account transmission of these organisms is easily produced experimentally in animals and according to Netter is not infrequently seen in man. Transmission has also been shown experimentally in animals with rabies and glanders (Loeffler[7]) and occurs in man in typhoid fever and variola. It has been especially discussed in tuberculosis, syphilis, and leprosy.

*Tuberculosis.*—Baumgarten[8] and his pupils attribute many cases of tuberculosis to placental transmission. The organisms do not cause disease immediately or soon after birth, but remain dormant a long time. At puberty antagonistic forces present in young and embryonal tissues against the inherited tubercle bacilli give out, and then the latent organisms begin to grow. Statistical evidence for Baumgarten's view is furnished by the fact that tuberculosis is abundant in the first and second years of life, and again from the beginning of puberty to the age of about thirty. The doctrine of *latent tuberculosis* is also supported by von Behring,[9] who believes that the tuberculosis is not inherited, but arises from an infection through the intestine in the period of suckling, remains latent for a long period, and then develops in adult life. The views of Baumgarten and von Behring have not been entirely accepted and there are many opponents to them. Thus Hauser[10] has shown on the basis of 1800 autopsies that malignant tuberculosis is common in very young children, and this frequency is evidence against any special resistance to tuberculosis in this period. Similar results have been obtained by Schmidt. Wassermann and Keysser state that not a single definite case of placental transmission of latent tuberculosis has thus far been demonstrated.

Experimentally the placental transmission of tuberculosis has been investigated on guinea-pigs and rabbits, but the results are by no means in agreement. Sanchez-Toledo[11] infected pregnant guinea-pigs with tubercle bacilli, 16 intravenously, 11 intrapleurally, and 9 subcutaneously. He made careful microscopic examinations of the liver, spleen, and other organs, took cultures from both spleen and liver, and injected emulsions of spleen, liver, and fetal blood into the peritoneal cavity of guinea-pigs. In not a single instance in 65 cases did this investigator find the tubercle bacillus. These observations have been confirmed by Cornet.[12] Hauser[10] also produced chronic tuberculosis in guinea-pigs by injection of the tubercle bacillus into the pleural cavity and allowed the animals to copulate, fourteen to eighteen days after the tuberculous process was established, with each other and with healthy animals. Of the 22 young which were subsequently born, 7 had tuberculous fathers and mothers, 3 had a tuberculous mother and a healthy father, and 12 had a tuberculous father and a healthy mother. The animals were kept under observation four to thirty-two months. Only one animal developed tuberculosis after a long time and this infection may well have been an acquired ingestion tuberculosis. Gärtner[13] obtained somewhat different results. He produced a kind of acute miliary tuberculosis in rabbits by intravenous inoculation, allowed them to copulate and obtained 51 young in 10 animals, the period between infection of the parents and the birth of the young being four to seventeen days. Of the young, 5 were found tuberculous by intraperitoneal injection of guinea-pigs. In mice he established lung tuberculosis by injecting the tubercle bacillus into the trachea and got 8 positive results in 57 experiments. Here the young were born fifty-six to two hundred and fifty days before the death of the mother. Gärtner concluded that with animals like mice and rabbits tubercle bacilli can pass directly from the mother to the fetus.

*Congenital Tuberculosis.*—Many cases of congenital tuberculosis, *i. e.*, tuberculosis of the fetus at the time of birth, have been reported, but the instances where tubercle bacilli have been found in the tissues or have been demonstrated by the injection of animals are very rare. Lebküchner[14] believes that of the 115 reported cases 15 may be accepted, and all of these may be ascribed to placental transmission. The principal lesions are in the liver and the adjacent lymphatic glands, those parts of the fetus

where the placental blood first lodges. While Baumgarten believes that the infection is hematogenous it must be remembered that the placenta has no immunity to tuberculosis, and can easily become infected.

Wassermann and Keysser[15] conclude that inherited tuberculosis can occur in animals by way of the placental circulation in heavily infected mothers, but is infrequent, only up to 10 per cent. of the offspring. In man it is extremely rare and occurs only in severe cases when the placenta itself is diseased.

*Leprosy.*—It was formerly believed that hereditary transmission was an important method of spread in leprosy, but there was little scientific evidence for the correctness of this view. Recently Zambaco Pacha[16] has pointed out that the children of leprous parents frequently become leprous, hence inheritance may play a rôle in the dissemination of the disease. Leprosy does not develop early after birth, but about the time of puberty. Von Düring[17] states that hereditary transmission is really negligible in leprosy since leprosy bacilli are present in the nose in 50 per cent. of the cases. The mucus containing the organism may be the infectious agent and the nose the portal of entry.

*Syphilis.*—Congenital lues, in which the fetus is syphilitic at birth, is far more common than congenital tuberculosis and hereditary syphilis is a relatively frequent occurrence. *Spirochæta pallida* grows well in fetal tissues, in larger masses than in adult tissues, especially in the liver. It is frequently present in the placenta and in the umbilical cord. Mohn[18] found it in the placenta in 70 per cent. of the cases where the parents were syphilitic and in the umbilical cord in 50 per cent. Furthermore in latent syphilis the spirochetes seem to have a special disposition to multiply in the organs active during pregnancy. It has been shown by Thomsen and Boas[19] that in patients with latent syphilis and a weak Wassermann reaction the colostrum gives a positive result. Massone[20] has found a stronger Wassermann in the serum from the placenta of a woman with latent syphilis than in the serum from other regions.

The syphilitic infection of the fetus is probably always due to a syphilitic placenta in the mother. There is no modern support for Colles' or Propheta's law that a luetic father can infect the ovum of the mother who becomes immune from the absorption of antibodies produced by the fetus. When a child is syphilitic from a syphilitic father, the mother always gives a Wassermann reaction whether she has symptoms or not, and her immunity is due to a latent infection. There is no evidence of *germinal infection* from the father. Rather the father infects the mother and the spirochetes travel through the placenta to the fetus. Furthermore the transitory immunity of the children born of a syphilitic mother is also due to a latent infection and Matzenauer[21] has shown that a positive Wassermann reaction occurs within a few days after birth.

**Germinal Transmission.**—At the time of ordinary acute infections there is comparatively little opportunity for the transmission of infection by way of the ovum or sperm since there is little chance of impregnation. Germinal transmission has been discussed chiefly in tuberculosis and has been especially emphasized by Baumgarten. Baumgarten's belief in this transmission rests chiefly upon the observation that tubercle bacilli may be found in the semen of man and animals with tuberculous testicles and that the disease may be localized primarily in regions of the body not communicating with the air, as in primary bone and joint tuberculosis and in adrenal tuberculosis. Such infections Baumgarten believes to be hematogenous in origin and usually latent. He traces them to a germinal infection. Tubercle bacilli

may be present in the testicles and semen of tuberculous men without lesions. Thus Jani[22] observed them five times in the testicles of tuberculous men without pathological anatomical alterations in the testicle. According to Simmonds[23] they are found in the sperm only in severe disease of the lungs with complications in other organs. They are also found in the sperm in animals with severe general tuberculosis.

Experimentally there is no evidence of germinal transmission of tuberculosis. Thus Gärtner produced tuberculosis of the testicles in 43 instances in rabbits and guinea-pigs and did not find a single case in the offspring of such diseased animals although genital tuberculosis might develop in the female. Cornet found no tubercle bacilli in 32 fetuses from 20 guinea-pigs with infected testicles.

In fowls the results are somewhat different. Maffucci[24] has shown that if eggs be artificially infected the birds from these eggs become tuberculous, twenty days to four and a half months after birth. Gärtner injected canaries intraperitoneally with human strains. He obtained 9 eggs from 12 canaries and 2 of these were found to contain the organisms by injection into guinea-pigs. The bacilli thus go into the eggs of birds with chronic abdominal tuberculosis. It has also been shown by Koch and Rabinovitsch[25] in spontaneous tuberculosis in birds, that among 118 infected the ovaries were diseased five times while tubercle bacilli were present in the eggs four times.

The conditions in birds in which the eggs carry the infection from mother to offspring are not strictly analogous to germinal infection in man. This type of infection really corresponds more closely to placental transmission.

At the present time we may say that definite proof of the hereditary transmission of tuberculosis by the sperm of the father has not thus far been brought.

In infections carried by insects the eggs may become infested with the parasite and the young transmit the infection. Koch[26] has shown that *Spirochæta Duttoni*, the cause of African recurrent fever, may be found in the eggs of infected ticks, *Ornithodorus moubata*, and Moeller[27] has proved the infectiousness of ticks up to the third generation by infecting apes and rats. Similar observations have been made on other spirochetal diseases.

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## Chapter IV

# NATURAL AND ACQUIRED IMMUNITY

It has already been pointed out that among the countless bacteria which have been investigated only a small number are endowed with the property of producing disease or inflicting injury in man and animals. These pathogenic organisms are widely distributed and would regularly invade the tissues and cause illness were it not for the defensive mechanism which the tissues exert against them. This defensive mechanism varies greatly in its strength and we find, in consequence, that there are natural limitations to infections. We express this fact by saying that certain individuals have a natural resistance or immunity to disease, or conversely, that certain individuals have a natural predisposition. Since most of our knowledge in this field relates to the factors by virtue of which the entry of micro-organisms and the invasion of the tissues is prevented or inhibited, we usually emphasize the resistance or immunity rather than the predisposition or susceptibility. Natural resistance or immunity varies according to the species, to the race, or to the individual, and we therefore speak of species resistance or immunity, racial resistance or immunity, and individual resistance or immunity.

## SPECIES IMMUNITY

Human diseases rarely occur spontaneously in animals. Typhoid fever and Asiatic cholera are widely spread in man and do not occur naturally in other species. Infectious processes not unlike typhoid fever and cholera may be produced in animals by way of the intestinal tract by a number of procedures, but not the natural disease. The resistance or immunity of animals to these diseases is, therefore, not *absolute*, but *relative*, depending somewhat upon the portal of entry. No animal is susceptible to leprosy and the leprosy bacillus does not grow in animals no matter how it be introduced. Animals thus have an *absolute* immunity to leprosy. Other diseases, like syphilis, influenza, and typhus fever, do not appear naturally among animals, but infections with the viruses causing these diseases can be produced, although with difficulty. Aside from leprosy and one or two other infections most examples of resistance in animals to human disease are examples of relative immunity. Man is susceptible to many diseases naturally affecting animals, like glanders, anthrax, and bovine tuberculosis. Infections with organisms belonging to the hemorrhagic septicemia group represented by the bacilli of swine-plague and cattle-plague, to which all animals are naturally susceptible in some degree or other, occur but rarely in man. Only one or two human cases of hemorrhagic septicemia from the members of this group have ever been reported, despite the fact that animals dying or dead of this infection are constantly handled by man, and the organisms must have abundant opportunity to produce infection. We may say that man has almost an absolute resistance to hemorrhagic septicemia. Mammals and birds do not exhibit the same susceptibility to disease. Fowls do not suffer from infections by the tubercle bacillus of man or cattle. This resistance is relative, since the introduction of large quantities of these organisms will produce disease in birds. Fowls have a natural immunity to tetanus. Another example of species resistance or immunity is that of dogs

and of rats against anthrax. Cold-blooded animals are naturally resistant to infection by pathogenic organisms producing disease in the warm-blooded, and infectious material from cold-blooded animals does not grow in man.

## RACIAL IMMUNITY

Instances of racial immunity to disease are rare and the immunity is only relative in most cases. Yorkshire swine are more resistant to swine-erysipelas than ordinary swine, and Prettner[1] claims that buffalo calves are more resistant to bovine tuberculosis than ordinary calves. Algerian sheep are more resistant to anthrax than other breeds. Holstein cattle are said to be more susceptible to tuberculosis than other varieties. Different races of mice differ markedly in their susceptibility to infection with the glanders bacillus and *Micrococcus tetragenus*, but this is a relative immunity, since infection can be produced with large doses. In man instances of racial immunity were formerly much emphasized. The Ethiopians were said to be naturally resistant to lues and malaria. This resistance is now explained on the basis of an acquired immunity from infection. There are several examples of racial immunity or susceptibility which cannot be so explained. Jews have in general a greater resistance to tuberculosis than other races, and this cannot be completely correlated with differences in the hygiene of living conditions. In the Southern part of the United States colored people seem to have a natural resistance to diphtheria, and this disease is less common among them than among the whites. The colored race apparently has a special susceptibility to tuberculosis.

## INDIVIDUAL IMMUNITY

In man different individuals show great differences in resistance to infection. Some persons have severe attacks of disease, some mild attacks, and some escape entirely. This is well illustrated in water-borne epidemics of typhoid fever where the water-supply is heavily polluted with fecal bacteria and must contain the typhoid bacillus. Only a certain proportion of the population using this infected water develops typhoid fever, and these cases show great differences in severity, although the infectious agent has presumably the same virulence and is, in all probability, distributed somewhat uniformly. The same holds true for Asiatic cholera, where only a certain part of the population acquires the infection in times of epidemics.

Among animals similar differences in resistance to infection are frequently shown. With an anthrax bacillus of low virulence only a certain number of rabbits succumb to artificial inoculation. Other species may not show this difference in susceptibility to anthrax, since all guinea-pigs die from an infection with this organism.

In general, the lower the virulence of the infectious organisms, the less difference shown in the resistance to infection of the different individuals in the same group.

## ACQUIRED IMMUNITY

Following an attack of an infectious disease those recovering lose their susceptibility to subsequent attacks. We speak of this condition as an *acquired immunity* to disease. This acquired immunity varies greatly with the infectious agent and somewhat with the individual. Thus all individuals develop a complete and lifelong immunity to smallpox after a first attack. Second attacks are so rare as to leave some doubt as to their occurrence. The immunity in yellow fever and bubonic plague is usually complete and lifelong. The majority of people develop a prolonged immunity to some of

the exanthems like measles, but here second attacks do occur, but rarely. It is usually believed that immunity develops after an attack of typhoid fever. This type of immunity may give out and subsequent attacks occur. With other infectious diseases the immunity which follows an attack is transitory. Comparatively little immunity is seen in pneumonia, where second and third attacks are not uncommon. In some types of infection certain people develop not an immunity, but a heightened susceptibility to this particular infection. This is seen occasionally in pneumonia and especially in streptococcus infection.

## ARTIFICIAL IMMUNIZATION

The theory that immunity to any disease can be artificially induced by deliberately producing attacks of this disease is apparently very old, for it is said that Mithridates was in the habit of immunizing himself to poisons by ingesting small quantities or by eating the blood of ducks fed on them. It is also possible that the snake charmers of India accustom or immunize themselves to the poisons of venomous snakes. In China and in India it was recognized in the 11th and 12th centuries that attacks of smallpox left behind an immunity which might last for many years and even for life. To produce a similar resistance to smallpox the disease was artificially induced by the priests of India, who dried the material from the smallpox pustules on silk threads and introduced it in the nose or beneath the skin. As a result genuine smallpox developed, lighter in character and far less fatal than the natural disease. This practice was widely prevalent in the Orient, and in 1718–1721 Lady Mary Wortley Montague, who had become familiar with it while living in Constantinople, introduced it into England. Here the ravages of smallpox were so great and attended with such a high mortality that artificial inoculation, or *variolation*, was largely followed. Variolation had a great vogue in America under Zabdiel Boylston, who must have had an excellent virus, inducing an immunity without giving rise to any appreciable mortality among the inoculated. Later Edward Jenner, a pupil of John Hunter, learned of the popular belief in Gloucestershire that immunity to smallpox followed an attack of cowpox, a disease common in England. Jenner inoculated a boy (James Phipps) with cowpox virus and subsequently tested his immunity to smallpox by inoculation with the virus of this disease. Jenner found that the protection against smallpox from cowpox virus was just as complete as that from the use of the genuine virus and, fortunately, realized the value of the discovery. As a result of Jenner's efforts the practice of *vaccination*, as it was called, in which cowpox virus was used for inoculation in order to prevent smallpox, became wide-spread, first in England and on the continent of Europe, then throughout the entire world. If we estimate the value of medical discoveries by the number of lives saved Jenner's discovery of vaccination may be said to be the greatest.

Following Jenner's observations no subsequent work of importance in this field was carried out till 1880, when Pasteur[2] discovered that attenuated cultures of the micro-organism of fowl-cholera would protect animals against the natural disease. Acting on this suggestion he later developed a practical method of protecting cattle and sheep against anthrax or splenic fever by the use of vaccines of the anthrax bacillus, the virulence of which was lessened by the action of heat. Subsequently Pasteur developed the method of protective inoculation against rabies, using in this case the virus in the spinal cord of rabid rabbits attenuated by drying over caustic potash. Vaccination against typhoid fever was then tried out in the British Army by Wright, who employed killed cultures of *Bacillus typhosus* in order to induce artificial

immunity. Since that time vaccines of great variety and mode of preparation have been utilized in cholera, dysentery, plague, pneumonia, streptococcus and staphylococcus infections, and in animal diseases like hog-cholera and cattle-plague. In no instance is protective inoculation so effective and successful as in smallpox, rabies, and typhoid fever.

This type of immunization, in which the infectious agents are used, is called active immunization to distinguish it from passive immunization. In the latter the artificial immunity is produced by transferring the blood-serum of human beings who have recovered from disease or of animals which have been actively immunized with bacteria or their products. It is especially employed in diphtheria, tetanus, and botulism. Animals are immunized with the soluble toxins which are produced by the diphtheria bacillus, the tetanus bacillus, or the botulinus bacillus, antitoxic sera being obtained and employed for therapeutic and prophylactic purposes.

## FUNDAMENTAL FACTORS OF IMMUNITY

Certain types of species immunity are easy to apprehend, since it is natural that an infectious organism highly specialized to grow in one species of animal would not develop in another. Bacteria which infect warm-blooded animals and grow readily at the temperature of the body are not likely to multiply in the body of cold-blooded animals. If the temperature of cold-blooded animals be raised they become susceptible to the infectious agent. Frogs, naturally immune to tetanus, develop the disease if their temperature be raised artificially. In this case the cold-blooded animals are no longer to be regarded as normal, since the artificial elevation of the temperature may bring about other changes which permit the development of micro-organisms. Again, the entire *metabolism* of one species differs so greatly from that of another that bacteria, which are highly differentiated to grow in one type, are not likely to find the proper condition in the tissues and in the blood-stream for their multiplication in another. The tubercle bacillus from man and cattle would not find the same conditions as to metabolism, chemistry, etc., in birds as in their natural habitat. In infections which take place through the intestinal tract certain species have a natural defense against the micro-organisms coming from other species in the different reactions of the intestinal juices and in the different enzymes of the alimentary canal. The high acidity of the dog's stomach suffices to kill off the ordinary bacteria which cause intestinal infections in man. Herbivora in general are not susceptible to infectious bacteria from carnivora, and this may be due to the destruction of bacteria by the intestinal enzymes or to a different metabolism. In other instances the resistance of one species against infectious material from another is not so easy to understand. Dogs are naturally resistant to anthrax and glanders, yet the etiological agents of these diseases multiply readily in both herbivora, like horses, and in omnivora, like man.

Finally, we have no adequate explanation at the present time for such instances of absolute immunity as are seen in animals against leprosy and in man against hemorrhagic septicemia. In *racial immunity* the resistance of one race for the infectious agents of another is equally obscure. It may depend on metabolism, which may differ markedly among the different races of the same species without our being able to detect it by chemical tests. In many instances racial immunity is to be regarded as an acquired immunity, and there is some evidence that this is inherited, as in the case of the immunity of Algerian sheep against anthrax.

In instances of *individual immunity* a great many factors protect the body

from invasion by bacteria of which only a few are understood. As we have already seen, the skin and mucous membranes offer almost complete protection against infection. In the *skin* this may be due to the anatomical character of the epithelium, the lack of moisture, and the acidity of the sweat. In the *nose* the ciliated epithelium tends to discharge micro-organisms eliminated in the nasal secretions. Bacteria introduced into the *conjunctival sac* are carried by the stream of secretions through the lacrimal duct into the nose. In the *mouth* the normal flora is well established and may prevent the development of parasitic forms. In the *bronchi* an outward stream of mucus is produced by the ciliated bronchial epithelium. The *lungs* are thus protected from infection by conditions existing in the eye, nose, mouth, and bronchi. In the *digestive tract* the acidity of the gastric juice may destroy some micro-organisms, while others would find difficulty in competing with the normal intestinal flora and establishing a foothold there. In the *vagina* the acid reaction may be important in preventing the multiplication of infectious organisms.

All these factors by means of which the individual is protected from infection may be altered and lost under abnormal conditions. Eye-strain and conjunctival irritation may alter the conjunctival secretions and predispose the eyes to infection. Altered reactions of the gastric juice and a lessening of the normal acidity may permit bacteria to survive which are normally destroyed, as in the case of Asiatic cholera where the incidence seems to be increased in people with gastric catarrh. Fatigue may predispose the body to infection; in chronic diseases like diabetes and pernicious anemia the natural resistance may be completely broken down and the body be subject to infections from various micro-organisms. Aside from the mechanical factors which protect the individual modern investigation has shown that many of the bacteria which enter the body are destroyed or rendered harmless under normal conditions. The internal organs not communicating with the external air are free from bacteria. As originally shown by Fodor[3] and Wyssokowitsch[4] the injection of large quantities of bacteria into the blood is followed by their disappearance without their going through the kidneys. The bacteria are actually killed in the blood-stream, and this destruction is the basis for the natural immunity which exists in many persons. Two main agencies are responsible for the destruction of bacteria, the *bactericidal* property of the blood-plasma furnished by the *alexins* and the activity of the cells, by virtue of which they engulf and destroy bacteria, or *phagocytosis*. It is impossible to differentiate sharply between the bactericidal action of the serum and the phagocytic action of the cells, and in life the two activities undoubtedly go hand in hand.

Several theories have been suggested to explain the *acquired immunity* which develops after an attack of disease or after protective inoculation. Both Pasteur and Klebs[5] believed in *exhaustion*, by which the infectious material used up the substances in the body necessary for the growth of the organism. Chauveau[6] had a different idea and assumed that the infectious agents left substances behind in the body which prevented the development of the same organisms when reintroduced. According to Metchnikoff the invading bacteria are engulfed by phagocytes just as in natural immunity, except that the immune body has a greater capacity for phagocytosis. After the discovery of *antitoxins* by von Behring and *immune bacteriolysins* by Pfeiffer it was recognized that the body elaborates *protective substances* during its recovery from disease; these protective substances remain in the tissues and blood for some time and the infectious agents are destroyed on their re-entry into the body, or made harmless by a neutralization of their

poisonous or toxic products. These substances are called "antibodies" and are not only an expression of acquired immunity, but are to be regarded as reaction products against the infectious agents. The formation of antibodies is closely related to the healing of disease. They are present in the blood-stream and may be transferred to other people, conferring protection on them. This is known as *passive immunity* as contrasted with *active immunity*, in which the body manufactures its own antibodies.

In many instances the presence of protective substances will not explain acquired immunity. An immunity to disease may exist without our being able to demonstrate any in the blood. After an attack of typhoid fever or cholera an immunity may exist for many years, yet the blood of these immune persons may show no more protective substances than that of the normal. Kolle[7] states that we cannot get away from the fact that in many infections we have, in addition to the immune materials, a specific biological change in the resistance of the tissues and cells for the infectious agent. It is possible that the immunized body reacts differently from the normal in its production of antibodies. The same irritation from the infectious agent which causes the normal body to produce a small quantity of antibodies may cause the immunized body to produce a large quantity in a short time. The body cells may have *learned* to manufacture antibodies and have an altered sensitiveness to stimulation. This condition may last for life, just as in the ganglion cells of the brain which have learned to react promptly to certain stimuli. Finally, there may be an *active local immunity* where the local cells have been trained to make antibodies quickly. Thus it has been shown by Wassermann and Citron[8] that antibodies are frequently assembled at points where infection takes place, that is, at the point where infectious material is introduced in artificial immunization. The local cells likewise may be so changed that they have a heightened phagocytic power to destroy cells when introduced. In acquired immunity several types of substances may be demonstrated in the blood and tissues, antitoxins, bacteriolysins, agglutinins, precipitins, and complement-fixing bodies. Some of these are to be regarded as distinctly *protective* in character, as the antitoxins. Others, like the precipitins, agglutinins, and complement-fixing bodies, have not been demonstrated to possess protective power, but are to be considered as reaction products resulting from the immunization with the infectious agent. Protective substances are also frequently present in normal people in small amounts, but they do not differ definitely in character from those found in the immune; they may be the result of a low grade immunization during which small quantities of infectious material are introduced from time to time without giving a noticeable reaction on the part of the body.

Finally a hypersensitiveness or hypersusceptibility to disease is seen after certain kinds of infection in man and animals, generally included under the heading of anaphylaxis.

## BACTERICIDAL ACTION OF BLOOD

The property of the extravascular blood, by means of which it destroys bacteria, was first observed by John Hunter who found that shed blood resists putrefaction. Fodor[9] subsequently demonstrated that anthrax bacilli go to pieces in the heart's blood of freshly killed animals. This work was elaborated by Nuttall[10] who found that anthrax bacilli, *Bacillus subtilis*, *Bacillus megatherium*, and *Staphylococcus aureus* disintegrate in the body fluids, defibrinated blood, pleuritic exudate, aqueous humor and the pericardial fluid of rabbits, mice, sheep, dogs, and pigeons. This bactericidal action was destroyed by heating to 56° C. Nissen[11] now showed that peptone blood

has a similar bactericidal action, and that the introduction of too many organisms into the extravascular blood or into the blood-stream does away with this bactericidal property. Buchner[12] found that the cell-free serum has the same bactericidal action as whole blood and called the bactericidal substances of the blood *alexins* (Gr. ἀλέξειν, to ward off or protect). The later work of von Behring and Nissen[13] demonstrated the specificity of the reaction and di Giaxa and Guarnieri found that the destruction of bacteria takes place in the blood in an isolated blood-vessel. Subsequently Tria[14] found a germicidal action in muscle juice, Prudden[15] in ascites and hydrocele fluid, Stern[16] in exudates and transudates. Finally a number of authors including Hankin, Kanthack and Denys have obtained bactericidal substances from leukocytes known as leukins.

The alexins of Buchner,[17] by virtue of which bacteria are destroyed and the body protected from the invasion of micro-organisms, possessed somewhat remarkable properties. They deteriorated in the presence of light and oxygen; were not injured by freezing and thawing; could be preserved for weeks in the cold, gradually losing in strength, however. They remained unchanged at 37.5° to 37.8° C. for twenty hours, were weakened at 44.8° to 45.6° C. in the same length of time, were destroyed at 50° to 51.5° C. in six hours and at 55° C. in one-half hour. They were destroyed or used up by contact with living bacteria or their decomposition products. They were not digested by pepsin and not injured by dilute acetic or sulphuric acid. Their action disappeared when the serum was diluted in 12 volumes of water, but remained intact on admixture with equal volumes of alkaline salt solution. They could be dialyzed with distilled water, but not with physiological salt solution. Finally they could be precipitated by 90 per cent. sodium sulphate solution, dried, and then taken up in water with their activity unimpaired. When dried they resisted the desturctive action of heat longer than when moist.

About this time it was shown by Pfeiffer[18] that if animals like guinea-pigs be immunized against the cholera vibrio, fresh organisms introduced into the peritoneal cavity are rapidly immobilized and dissolved. This phenomenon is known as "Pfeiffer's reaction" or "Pfeiffer's phenomenon." Further analysis of this reaction revealed that it also occurred in the test-tube with fresh immune serum, but this immune serum soon lost its property of dissolving the vibrios. This property was also done away with by heating the serum to 57° C. half an hour. On the addition of fresh normal serum from the same animal or from another species the capacity of dissolving the vibrios was restored to the immune serum. There were thus two components demonstrable in a cholera immune serum both of which took part in the immune bacteriolysis known as Pfeiffer's reaction. It had long been known that the blood-serum of one species of animal was poisonous when introduced into the circulation of another species and that this poisonous action depended on the property which certain bloods had of dissolving the corpuscles of others. The resemblance of *hemolysis*, as this property of the blood was called, to *bacteriolysis* was suggested by the work of Doremberg[19] who proved that the hemolytic property of blood was destroyed at 50° to 55° C. Subsequently Belfanti and Carboni[20] found that this property depended upon two substances, one of which was destroyed by heat while the other resisted this action. It was later shown by Bordet[21] that a *hemolytic immune* serum could be produced in animals by the introduction of alien blood corpuscles and this serum closely resembled a bacteriolytic immune serum. The properties of normal and immune hemolytic and bacteriolytic sera were subsequently investigated at great length by Bordet and by Ehrlich and Morgen-

roth[22] and there is now general agreement as to their essential mode of action. The thermolabile constituent of all types of bacteriolytic serum was called "substance bactericide" and the thermostable component, "substance sensibilisatrice." According to Bordet the former substance was furnished by the normal and immune body and was the same in hemolysis and bacteriolysis. It was identified by Bordet as Buchner's alexin. This thermolabile substance was called "complement" by Ehrlich and Morgenroth who originally believed that a different kind of complement was active in the different types of bacteriolysis and hemolysis which occurred naturally and could be produced artificially. Subsequently it was shown by Bordet and Gengou,[23] by the reaction known as "complement fixation," that the "substance bactericide" or complement is identical in normal and immune sera and this has been practically confirmed. Complement may be regarded as equivalent to Buchner's alexin. It must be remembered that it represents only the thermolabile component of the alexin, which consisted of all the substances of the normal sera active in the destruction of bacteria and really corresponded to what we now designate as a normal bactericidal or bacteriolytic serum.

The thermostable component of normal and immune bacteriolytic sera was designated by Bordet as "substance sensibilisatrice" and by Ehrlich and Morgenroth as "amboceptor," "immune body," or "antibody." It is agreed that this is the variable component of the serum, is specific in action and has affinities for particular types of blood-cells or bacteria against which man or animals may be immunized either artificially or naturally.

Other nomenclature for these substances is occasionally employed. Thus Metchnikoff calls the complement "cytase" and the immune body "substance preventive."

## PROPERTIES OF THE IMMUNE BODY

The thermostable component of an immune serum, called "substance sensibilisatrice" by Bordet and "immune body" by Ehrlich, is highly specific in action and is related to the particular types of cells with which the animal is immunized. A cholera immune body has no action on the typhoid bacillus and a typhoid immune body has no action on the cholera vibrio. A hemolytic immune body has no relation to a bacteriolytic immune body. The relations of the immune bodies are complicated, however, since they occasionally act upon closely related types of cells. An immune body made by injecting an animal with the typhoid bacillus has some action on the closely related paratyphoid bacilli. Similar affinities may be seen in hemolytic immune bodies.

The immune body combines with its corresponding antigen. This may be demonstrated by putting mixtures of antigen and immune body on ice and after a short time it can be shown that the immune body is completely absorbed in the antigen. A mixture of blood-cells and hemolytic immune body remains intact till the addition of complement when the cells are rapidly dissolved.

Little is known about the chemical composition of the immune bodies. Pfeiffer and Proskauer[24] demonstrated that the bacteriolytic immune bodies are not protein, albumoses, or peptone. Thus a cholera immune serum may be repeatedly taken up in alcohol and dissolved in water, the final product being rich in cholera immune bodies, but protein-free as far as chemical tests are concerned. Huntoon, Masucci, and Hannum[25] have obtained pneumococcus antisera which do not give protein color reactions and do not contain sulphur or phosphorus. These authors believe that antibodies do not belong to the group of proteins usually classed as serum proteins.

## PROPERTIES OF COMPLEMENT

Complement has many of the properties originally ascribed to Buchner's alexins. It can be preserved at low temperatures and resists drying. It is destroyed by exposure to a temperature of 56° to 57° C. for half an hour. It is a normal constituent of the blood-serum, of the body fluids, and of certain tissues and cells. The amount of complement varies with the species of animal and certain types like guinea-pigs have a uniformly high amount in their serum. We know little about its chemical nature, but its sensitiveness to heat and its relation to salt suggest that it may be a protein. Certain kinds of complement like hemolytic complement show a curve of reaction like that of proteolytic enzymes and many authors believe that hemolytic complement is certainly an enzyme. While the complete identity of hemolytic and bacteriolytic complement may not be established with certainty, there is sufficient parallel in their action to make us believe that if hemolytic complement is an enzyme bacteriolytic complement is also one. According to von Liebermann[26] hemolytic complement is not a ferment, but a soap, also inactivated at 55° C. Landsteiner and Ehrlich believe that bactericidal complement may be a lipoid-like substance which attacks the fat-like bodies in the bacterial cells in bacteriolysis. Hahn[27] states it has not been demonstrated that all bacteria have a lipoid membrane, and proof has not yet been brought that lipoids in bacteria are injured in bacteriolysis.

Complement exists normally in the circulating blood and its presence may be of the greatest importance in warding off infection in adults. Embryonic tissue usually contains none. The amount in the blood diminishes just before death in fatal infections, as was first shown by Longcope and subsequently confirmed by Conradi[28] and Wilde.[29] Complement varies greatly in amount in the blood of individuals from time to time (Tromsdorf[30]) and this is a suggestive explanation for the variations in individual natural resistance. The ability of the body to resist infection is very complicated, however, and is based upon a number of factors of which the bactericidal action of the serum is only one. The source of the complement in the body has never been made out definitely. According to Metchnikoff it is always derived from the living leukocytes and is never free in the blood-serum, being liberated when the blood is clotted. Bacteriolysis is thus always the result of the destruction of leukocytes and not a true blood-serum phenomenon. Recent investigations indicate that complement is a complex substance consisting of at least three portions, the so-called mid-piece, the end-piece, and a third or heat resistant component (Hyde[31]). It may be divided by dialysis or globulin precipitation into a globulin fraction and an albumin fraction. The globulin fraction attaches itself directly to the cells sensitized or laden with amboceptor and is therefore called the mid-piece. The albumin fraction acts upon the combination of the cells with the mid-piece in the globulin fraction and is therefore called the end-piece. The third component reacts with the other two in the combination which makes up complement. Hyde has shown that the third component is inherited as a simple Mendelian recessive unit.

## THEORIES OF BACTERIOLYSIS AND HEMOLYSIS

Several theories have been suggested to explain the phenomena seen in serum hemolysis and bacteriolysis, but the only ones which have received any wide acceptance are those of Bordet and Ehrlich. Bordet believes that the thermostable component of an immune bacteriolytic (or hemolytic) serum has a kind of physical or physicochemical action on the cells employed in

immunization, rendering them susceptible to the influence of the bactericidal substance present in normal serum. This action Bordet likens to that of a mordant which affects cells in such a way that they can be stained later by certain dyes. By this theory the cells must first be "sensitized" by the amboceptor or "substance sensibilisatrice," after which they are killed and dissolved by the bactericidal substance of the serum, the alexin, or complement.

By the theory of Ehrlich, known usually as the "side-chain" or "lateral-chain" theory, the immune body or amboceptor, the complement, and the cellular antigen form a chemical rather than a physical combination or union. Ehrlich conceives of the protoplasm of the body as made up of a kind of working nucleus (Leistenkern), with certain lateral appendages which he calls receptors or haptines. Receptors of various kinds exist in aggregates of protoplasm. Some of these receptors can combine chemically with simple nutritive materials and serve for the nutrition of the cell. In order that the combination occur these nutritive materials must have atom complexes which attach themselves to the side-chains or receptors in much the same manner as a key fits into a lock. In addition to ordinary receptors higher types exist which anchor highly complex molecular protein which cannot be assimilated directly, but must first be converted or broken down by fermentative processes. These higher types of receptors possess fermentative groups called by Ehrlich zymogenic. Finally, the receptors are represented by Ehrlich as having certain complexes by means of which these various combinations with food material and higher protein are effected. These are known as *haptophoric groups*. Only these molecular complexes can combine with protoplasm which must also have haptophoric groups; the two sets of haptophoric groups thus effect the union of the protoplasm with the substances with which it comes in contact.

To explain the formation of immune substances Ehrlich assumes that the bacteria, which enter the body in natural infections or are introduced artificially, have atom groups or receptors which serve to anchor the nutritive material necessary for their development and other atom groups or receptors, which combine with the receptors of the protoplasm. The bacterial receptors are thus anchored to the cell receptors by means of the haptophoric or catching arms of each group of receptors. The receptors of the protoplasm, which have thus combined with the bacterial receptors, lose their natural function of taking up nourishment and the nucleus or Leistenkern of the protoplasm is correspondingly injured. To compensate for this injury the nucleus or Leistenkern manufactures new receptors of the same kind as those put out of commission by the bacteria. These new receptors are not formed in just the amount needed by the cells, but in excess, in accordance with the law, originally pointed out by Weigert, that after injury cells are always formed in superabundance. Since the receptors are no longer needed by the protoplasm and have no natural function they are given off to the blood. They correspond to the antibodies of Pfeiffer, and were named "amboceptors" by Ehrlich because of their dual combining capacity, by virtue of which they are related to the bacteria on the one hand and the body protoplasm on the other. When these receptors are attached to the protoplasm they combine with the bacteria and protect it. When free in the blood-stream they also combine with the bacteria and prevent the attack of the bacteria upon the protoplasm represented in the body cells. Ehrlich and Morgenroth regard the combination between the amboceptor and the receptors of the bacteria in bacteriolysis as a chemical combination. The bacteria are not dissolved as a result of this, but a third element effects the solution, the alexin or com-

plement, and this reaction is also chemical. In this conception the complement does not combine with the bacteria or necessarily with the amboceptor. Rather it combines with the amboceptor after union of this with the bacteria and then effects the destruction or the solution of the micro-organisms. The complement is not a single substance, but there are complements of great variety, bacteriolytic and hemolytic, each one of which is able to combine with an appropriate amboceptor and bring about bacteriolysis or hemolysis. The appended sketch illustrates graphically the mechanism by means of which bacteria combine with the protoplasm, injure it, and cause it to throw off the receptors in excess, the amboceptors or immune bodies.

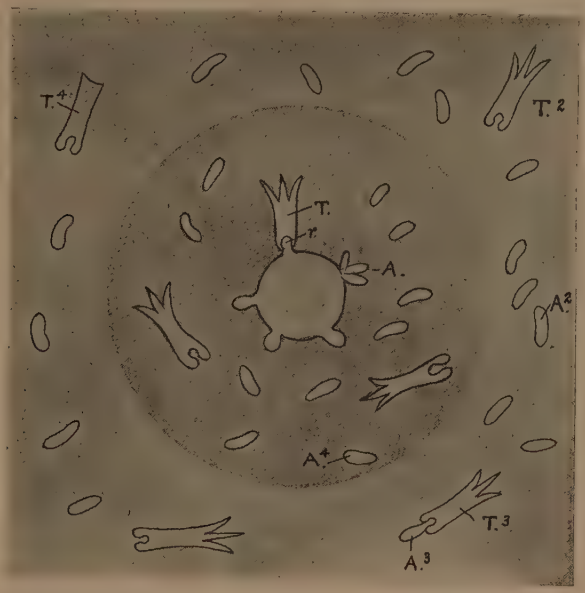


Fig. 176.—Theoretic formation of antitoxins. The central white area represents a molecule of a cell; the shaded portion represents the cell itself; the surrounding area represents the body fluids about the cell. r, A receptor of the molecule (*first order*); A, overproduction of receptors, which are being cast off; $A^2$, a cast-off receptor free in the body-fluids—now an antitoxin; $A^3$, a molecule of antitoxin in combination with a toxin molecule $T^3$. $A^3$, a cast-off receptor still within the parent cell; T, a toxin molecule in combination with the receptor of a cell molecule; $T^2$, a toxin molecule free in the body fluids; $T^3$, a toxin molecule in combination with antitoxin; $T^4$, a molecule of toxoid (toxophore group lost). (Kolmer.)

In this brief consideration of Ehrlich's side-chain theory, which has had an enormous influence in stimulating work in immunity and has helped greatly to clarify our ideas on this subject, it should always be remembered that the graphic illustrations and the various names employed by Ehrlich are not to be taken too literally. They are rather to be interpreted as a means of giving us a conception of the complex processes which must take place in the protoplasm of the cell as it is attacked or irritated by bacteria. The extent to which the Ehrlich theory has been confirmed by actual observation and experiment is also somewhat doubtful. The main tenet upon which it rests, namely, that the cells injured in infections are the cells or groups of cells which make antibodies or immune bodies, has not been satisfactorily proved. In many instances the immune bodies are not produced by injured cells, but rather by the hematopoietic organs of the body like the bone-marrow

and spleen. In isolated cases where antibodies appear locally at the site of infection injured cells may possibly give rise to them, but here it is also somewhat problematic.

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## Chapter V

## PHAGOCYTOSIS

METCHNIKOFF[1] and the large school of his followers find the chief defense of the body against the invasion of bacteria in the cellular elements of the blood-stream and of the tissues, in both natural and acquired immunity. The rôle of the white cells of the blood in freeing the body of pathogenic organisms was first recognized by Panum[2] and Roser,[3] but the immense importance of the activity of these cells was first adequately realized by Metchnikoff. Since all these cells can throw out ameboid protoplasmic processes and are able to engulf foreign bodies they were designated phagocytes (Gr. φαγεῖν, to eat, κύτος, a hollow cell). The phagocytic activity of the white cells is easily demonstrated in both plants and animals. If one injects foreign blood-corpuscles or foreign cells into the peritoneal cavity of an animal the number of leukocytes there first decreases slightly then undergoes a great increase and various types of cells may be found. These cells ingest the foreign elements which may be seen actually disintegrating inside of them. Two types of leukocytes are especially active in this phagocytosis, the large mononuclear cells which Metchnikoff designates *macrophages* and the polymorphonuclear neutrophils which he calls *microphages*. The macrophages are most active and are able to imbibe and digest large numbers of cells and cause them to disappear. This intracellular digestion is traced by Metchnikoff to an unorganized enzyme in the *macrophages* which he calls *cytase*. Such a cytase can be obtained from lymph-glands, spleen, and adrenals, organs which contain mononuclear leukocytes in great numbers. An extract of these organs will dissolve the red blood-corpuscles of other species and loses its activity when heated to 56° C. for half an hour. This macrophage extract is identified by Metchnikoff with a similar substance in the blood and is called *macrocytase*. It is also present in several exudates.

The cytase which Metchnikoff finds in the macrophages is probably identical with what is now described as complement or alexin, although there has been considerable discussion in regard to this point. Korschun and Morgenroth[4] have shown that the hemolytic substance of organs containing macrophages like the lymph-glands not only stands heating to 56° C., but is not destroyed by boiling. It is soluble in alcohol and is thus different from the true complement or alexin. Levaditi[5] has found, however, that if one takes fresh lymph-glands from guinea-pigs and tests their hemolytic strength immediately, the process goes on in just the way which Metchnikoff has described, the ability to dissolve red blood-corpuscles being lost when the extracts are heated to 56° C. In older lymph-glands hemolytic substances can also be obtained by maceration in salt solution for an hour. These are thermostable and are believed by Levaditi to differ chemically from the true cytase, being possibly amino-acids, fats, fatty acids, or soaps.

The cells designated macrophages by Metchnikoff include also certain connective-tissue cells, endothelial cells, those of the splenic pulp and certain ones of the lymph-nodes and neuroglia tissue. They are especially concerned in the ingestion of the foreign particles and detritus, and in many instances the infectious bacteria. The polymorphonuclear neutrophils of the blood, also active in phagocytosis, are called microphages by Metchnikoff.

They are abundant in pus where they are often designated "pus-cells." In general the microphages are more concerned with the ingestion and destruction of bacteria than are the macrophages. This is easily observed in pus where the ingestion and destruction of bacteria by the leukocytes is a common phenomenon. The bacteria taken up by the phagocytes gradually lose their shape, their staining properties disappear, and eventually nothing remains but a little detritus which may or may not take up bacterial dyes. There is, however, a good deal of variation in the fate of different bacteria. Organisms like the tubercle and leprosy bacillus retain their morphology and staining properties a long time inside the phagocytes, a month or more, and even after the morphology is lost acid-fast material may still be demonstrated inside the cells (Margaret Reed Lewis[6]). Spores remain intact for a long time. The digestion of the bacteria inside the microphages is also supposed to be due to an enzyme, not yet proved to be identical with the cytase of the macrophages.

The ingested bacteria are usually killed and dissolved in phagocytosis. This is not always the case, since the tubercle bacillus is not always killed in phagocytosis, and may increase in number and kill cells which have ingested it.

Metchnikoff further differentiates phagocytic cells into "wandering cells" and "fixed cells." In the circulating blood various cells may act as phagocytes, the large mononuclear lymphocytes, the polymorphonuclear neutrophils, the eosinophils to a much lesser extent. The small mononuclear leukocytes and the so-called "mast cells" have little or no activity. In the tissues the most active phagocytes are the macrophages and considerable discussion has been heard in regard to their origin. According to Mallory they are of endothelial origin. Sabin believes that only part of them, the clasmatocytes, are derived from the endothelium. Maximov traces the macrophages to the blood-cells and this is the view of the Lewises who have confirmed and extended this idea by cultures of blood. Similar macrophages are found in lymph-nodes, spleen, and liver. Zinsser and Carey[7] have observed phagocytosis of leprosy bacilli in cells from artificially cultivated splenic pulp in rats, probably connective tissue cells.

Phagocytes are not able to ingest bacteria immediately except in a few cases. As will be seen later infectious micro-organisms must be prepared for ingestion by the action of certain serum constituents called opsonins or bacteriotropins, or by immune bodies, called "fixateurs" by Metchnikoff.

The relationship of phagocytosis to natural immunity is now pretty generally accepted and it is believed that the phagocytes play the chief part in freeing the body from foreign particles of every sort, especially in the healing of infections where bacteria are taken up and digested in the cells. In animals naturally sensitive to anthrax infections like guinea-pigs and mice there is little phagocytosis of the anthrax bacillus whereas in animals naturally immune to this infection, like dogs, hens, and frogs, the phagocytosis of the organism is very marked. With an anthrax bacillus of low virulence phagocytosis does take place in a sensitive animal and it must be recognized that the virulence of the organism in infectious processes is intimately associated with its power of resisting phagocytosis. Denys and Marchand[8] have found that virulent streptococci are phagocyted only when *specific* serum is present while non-virulent organisms are phagocyted by *normal* serum and leukocytes. In tuberculosis the resistance to infection is dependent to some extent at least on the macrophages represented by the epithelioid cells of the tubercles. The natural immunity of guinea-pigs against *Spironema Obermeieri*, the organism of recurrent fever, is traced by

Sawtschenko and Melkich[9] to phagocytosis, as well as the resistance of guinea-pigs to the cholera vibrio introduced into the peritoneal cavity. Phagocytosis may be observed in natural immunity against such bacteria as the gonococcus, pneumococcus, staphylococcus, and streptococcus.

The number of cells in the body which can act as phagocytes in *natural immunity* is very large. It includes chiefly the mononuclear and polymorphonuclear leukocytes of the blood and lymph which may be regarded as wandering cells. Certain *fixed* cells like the so-called "dust cells" of the lung alveoli have a phagocytic action, but Tchistovitch[10] regards these cells as mononuclear leukocytes which have wandered in to the lung structure. The Kupffer cells or star cells of the liver may also be active phagocytes, but these again may not be fixed, but large mononuclear leukocytes which have wandered in (Margaret Reed Lewis). The large cells of the splenic pulp, of the lymph-glands, and connective tissues are the chief representatives of the "fixed macrophages." Phagocytosis is probably a more important mechanism for protecting the individual against infection in natural immunity than bacteriolysis; a large number of different infectious organisms are phagocyted while a comparatively small number are disposed of by bacteriolysis, which is somewhat limited in its action. It must be remembered also that phagocytosis can vary greatly in different persons and in the same person under different circumstances, just as other immune mechanisms vary greatly in their strength or activity from time to time.

In *acquired immunity* phagocytosis may play an important part, but the extent to which it is active in warding off infection in individuals who have recovered from disease is by no means clear. It has been shown by Metchnikoff that in rabbits, guinea-pigs, and mice immunized against anthrax, phagocytosis is more marked than in non-immunized animals. No loss in the virulence of the organisms is seen in such cases. In rats immunized against anthrax the phagocytosis of *Bacillus anthracis* is increased. There is, in general, a rapid collection of phagocytes in the areas where the infectious bacteria are introduced, and then a rapid ingestion and digestion inside these phagocytes. Metchnikoff believes that this is accomplished by the *microcytase*, the digestive ferment from the microphage, and the "fixateur" which prepares the organisms for digestion by the leukocytes. The fixateur may be present in normal animals and is found constantly in immune animals. It originates, according to Metchnikoff, in the leukocytes, and thus a heightened phagocytosis came to be regarded as playing the chief rôle in acquired immunity. It is now recognized that a heightened phagocytosis may occasionally be observed in acquired immunity, but this is probably only a part of the heightened cell activity throughout the body.

Acquired immunity against infectious organisms probably depends primarily upon the presence in the blood-stream of bodies like bacteriolysins or the "substance sensibilatrice" of Bordet, which combine with bacteria and make them sensitive to the action of alexin, or upon immune opsonins or bacteriotropins, which act upon bacteria and render them susceptible to phagocytosis. The body described as "fixateur" by Metchnikoff, which he believed to be increased in artificial immunity, is frequently regarded as identical with the "substance sensibilisatrice" of Bordet, the amboceptor or immune body of Ehrlich. This identification assumes that phagocytosis and bacteriolysis are essentially due to the same substances, the alexin or complement being the same as the cytase from the leukocytes. Final proof has not yet been brought that the leukocytes are the source of alexin, and for the present we must regard phagocytosis and bacteriolysis as different phenomena.

It has already been pointed out that in guinea-pigs immunized against the cholera vibrio fresh organisms are rapidly dissolved in the peritoneal cavity (Pfeiffer's phenomenon) and this action is now attributed to the presence of bacteriolytic amboceptor and alexin or complement. Phagocytosis of the injected organisms cannot be observed. Metchnikoff thinks that this failure to demonstrate phagocytosis in Pfeiffer's reaction is due to the destruction of the phagocytes by phagolysis, so that the digestion of the vibrio takes place outside the cells. If the phagocytes in the peritoneal cavity are protected from destruction one can observe a true phagocytosis in which the vibrios are taken up by the leukocytes and digested. Phagolysis can be prevented by the intraperitoneal injection of broth or physiological salt solution. While phagocytosis can undoubtedly be observed in the peritoneal cavity of immune guinea-pigs after injection with broth or salt solution it must be remembered that this treatment results in the accumulation of large numbers of leukocytes in the peritoneal cavity of both normal and immunized animals, and these leukocytes would undoubtedly act on the organisms which are introduced. There is no proof that true bacteriolysis does not occur at the same time or is not merely prevented from occurring because of the presence of the phagocytes (Prausnitz and Hille).

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## Chapter VI

## OPSONINS AND BACTERIOTROPINS

Following the fundamental work of Metchnikoff on phagocytosis it was shown by Denys and Leclef,[1] by the study of the same phenomenon in the test-tube, that leukocytes from animals immunized against streptococci do not ingest these organisms more actively than normal leukocytes. It had previously been believed by Metchnikoff that the leukocytes themselves were more actively phagocytic in artificial immunity, being made so by the presence of bodies in the immune serum known as "stimulins," which acted directly upon them. From the observations of Denys and Leclef the increased phagocytosis was attributed to substances in the immune serum which combined with the bacteria, altering them so that phagocytosis took place. This phagocytosis was equally active with the leukocytes of a normal and immune animal.

Subsequently, Wright and Douglas,[2] employing Leishman's method of observing phagocytosis in a drop of human blood, studied the phagocytic action of serum and leukocytes against a number of infectious agents, staphylococci, gonococci, tubercle bacilli, and typhoid bacilli. These investigators discovered that fresh normal serum as well as immune serum so alters bacteria that phagocytosis becomes possible. The substances with this property were shown to unite directly with the bacteria and were *thermolabile*, being destroyed at 55° to 60° C. in ten to fifteen minutes. These thermolabile bodies, which were present in both normal and immune serum, were called *opsonins* by Wright (from Gr. ὀψωνεῖν, to prepare food for) and were further differentiated into normal and immune opsonins.

Finally, Neufeld and Rimpau[3] showed that substances are present in the blood-serum of animals immunized against streptococcus and pneumococcus which prepare these bacteria for ingestion by leukocytes. The bodies of Neufeld and Rimpau were *thermostable*, and could thus be differentiated from the thermolabile opsonins of Wright. They may be the same as the substances found by Denys and Leclef in streptococcus immune serum. They were designated *bacteriotropins* by Neufeld and Rimpau.

The opsonic content of the blood varies greatly in different people, and abundant possibility of clinical application, especially in vaccine therapy, seemed to be offered from its determination. The amount of opsonin was easily estimated by counting the bacteria taken up by the leukocytes after they were acted upon by various types of serum. This was called the "opsonic index." The variations in the opsonic index of normal persons are so great and the technic of its determination subject to so many difficulties that the sanguine predictions of its value in showing the resistance of different persons to infection have not been fulfilled.

The relationship of opsonins and bacteriotropins to each other and the part they play in natural and acquired immunity have been much discussed, and there is still considerable doubt as to their constitution. In general, it has been shown that *normal opsonins* play an important rôle in *natural immunity* and aid in the protection of the body by altering bacteria so that they can be ingested by phagocytes. They act upon a larger number of bacteria than are affected by the bactericidal substances in the blood,

especially upon the cholera vibrio, typhoid and dysentery bacilli, *Bacillus coli*, pneumococcus and staphylococcus, plague and diphtheria bacilli. They are present in the blood and lymph, in exudates, in the lacteal secretions. *Immune opsonins* are present in the blood in several infections. They have been found in typhosus serum by Levaditi and Inmann,[4] in dysentery staphylococcus and typhosus serum by Dean,[5] in human tuberculosis by Caulfield,[6] in diphtheria by Sauerbeck,[7] von Glahn and Ohkuro,[8] and in antidiphtheritic serum by Lindemann.[9] They are also present in anti-erythrocyte serum along with amboceptor and complement, as has been shown by Neufeld and Bickel.[10] How important they are in acquired immunity is still disputed, especially by Neufeld.

The constitution of opsonins is by no means easy to define. They are present in staphylococcus, plague, and Malta fever sera, which have no bactericidal power, as has been shown by Wright and Douglas and by Wright and Windsor. Hektoen has also found that anthrax bacilli, streptococci, and pneumococci are not killed in a strong opsonic serum. Normal and immune serum have a strong opsonic action against the tubercle bacillus, but no lytic action. Opsonins are abundant in antidiphtheria serum and bactericidal action is lacking. Opsonins thus cannot be identical with bacteriolysins. They are thermolabile like alexin or complement, which plays some rôle in the opsonin reaction. Serum loses its opsonic power hand in hand with its complement. Complement has been shown to be present in the opsonin reaction by Muir and Martin[11] and by Neufeld and Hüne.[12] Opsonin is also fixed or deviated by specific complement-fixing serum. According to Hektoen[13] and Ruediger[14] opsonins have the same constitution as agglutinins, being receptors of the second order in Ehrlich's nomenclature, with a haptophor and opsonophor group. Cowie and Chapin[15] have shown by absorption at 0° C. that the staphylococcus opsonin of normal serum has two components like amboceptor and complement. The same thing has been demonstrated by Meyer[16] for normal paratyphosus opsonin and by Hektoen for hemopsonin. The opsonin amboceptor in normal serum is specific, while the opsonin complement is not. Finally, it has been pointed out by Dean that some of the normal opsonins are not absolutely thermolabile and that bacteria treated with normal serum are prevented from taking up immune opsonins. The normal and immune opsonic substances may thus be identical, at least in part. The opsonic reaction also varies somewhat with the virulence of the bacteria. Hektoen[13] and Rosenow[17] have shown that avirulent streptococci and pneumococci are opsonized on the addition of normal serum (opsonin), while virulent strains are not so affected.

The *bacteriotropins* of Neufeld and Rimpau are now regarded as specific antibodies which are not destroyed or robbed of their activity by ordinary temperatures of inactivation, 55° to 60° C., act on bacteria without complement, and do not kill or visibly alter them. Like opsonins, they prepare the bacteria for ingestion by phagocytes. Bacteriotropins against the cholera vibrio and typhoid bacillus may be heated to 62° to 63° C. without destruction. They have been described in antidiphtheria serum by von Glahn and Ohkuro and by Lindemann. They possess the same degree of specificity as other immune bodies. Thus the paratyphosus tropins act on the entire paralyphoid group as well as on *Bacillus typhosus*. Tropins also occur for bacteria in inactive normal sera. They have been demonstrated for mouse typhoid (*Bacillus typhi murium*) and staphylococcus by Neufeld and Hüne, for *Bacillus typhosus* by Clark and Simmonds,[18] for *Bacillus anthracis* by Hektoen.

Tropins for blood-corpuscles or hemotropins have been described by

Sawtchenko[19] and by Hektoen. These may be heated to 70° C. without destruction. These hemotropins are not identical with hemolytic amboceptor and complement. They may be of inportance in diseases like typhoid fever and pernicious anemia where there is considerable phagocytosis of red blood-cells, which may previously have been "tropinized" by toxins. Hemotropins may occur in normal serum, as has been shown by Neufeld and Töpfer.[20]

Tropins are apparently relatively simple bodies like precipitins and agglutinins. They are not identical with bacteriolysins, since many immune sera, like those of streptococcus, pneumococcus, and glanders, contain tropins and yet have no lytic amboceptors, and, furthermore, they act without complement. They are not absorbed by complement-fixation serum. Dean and Hektoen point out some evidence to indicate that they are identical with immune opsonin, that is, with the amboceptor component of immune opsonin. This is not accepted by Neufeld,[21] who regards the bacteriotropins as the essential protective substance in immune serum which makes bacteria capable of undergoing phagocytosis.

**Spontaneous Phagocytosis.**—In certain exceptional instances leukocytes may take up unchanged bacteria, that is, bacteria which have not been acted on by opsonins. Löhlein,[22] Dean, and Hektoen have shown that bacteria are occasionally ingested by leukocytes when complement is not present to furnish opsonin. The mechanism of this phenomenon is not clear, but it may be related to the virulence of the organisms. Avirulent pneumococci may be ingested by phagocytes directly, while virulent pneumococci require the previous action of serum. Occasionally highly virulent organisms may be phagocyted directly, as with the tubercle bacillus and with non-encapsulated anthrax bacilli. Certain bacteria, like the diphtheria bacillus, are so subject to spontaneous phagocytosis that a specific action is difficult to demonstrate in serum.

**Bactericidal Substances from Leukocytes.**—The bactericidal substances of the blood-serum were regarded by Hankin and Wesbrook[23] as related to the granules of certain white blood-cells known as "pseudo-eosinophiles" or "amphophiles," which were looked upon as the mother substance of alexin. It was subsequently shown by Buchner and Schuster and by Hahn that polymorphonuclear leukocytes can be obtained in large quantity by the injection of aleuronat, glutin, and casein into guinea-pigs, rabbits, and dogs. These leukocytes may be destroyed by freezing and thawing and after digestion at 37° C. have as powerful a bactericidal action as the blood-serum of the animal from which they are derived. Bactericidal substances have been obtained from such leukocytes by Schattenfroh, by Van de Velde by extracting them with cold water, and by Bail by treatment with the "leukocidin" obtained from *Staphylococcus pyogenes aureus*. They are now usually called *leukines*.

The question of the identity of *leukines and alexins* has been much discussed. According to Schneider, Zinsser,[24] Weil,[25] and Petterson[26] the bactericidal substances from leukocytes are not identical with serum alexins. They are not rendered inactive by temperatures under 80° C., and when once inactivated by higher temperatures cannot be reactivated by the addition of fresh normal serum or of unheated leukocytic extracts. It has, furthermore, been shown by Petterson and by Zinsser that these bactericidal substances are not increased in immunization, the quantity in each leukocyte remaining fairly constant. They are called "endolysins" by Petterson. It has finally been shown by Petterson that leukocytes contain in addition to the heat-resistant bactericidal substance which is soluble in alcohol, another

substance which is soluble in alcohol and alcohol-ether, acts in aqueous solution, and is not heat-resistant. The exact nature of this substance has not been determined.

We may therefore say that polymorphonuclear leukocytes certainly give off bactericidal substances which are different from true serum alexins. It is not yet determined with certainty whether they give off true alexins.

Extracts of microphages are neither bacteriolytic nor hemolytic, although both bacteria and red blood-cells go to pieces inside them.

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## Chapter VII

## ARTIFICIAL IMMUNIZATION

In the production of specific immunity we must distinguish between an immunity against the living infectious material, bacteria or protozoa, that is, a *resistance to infection*, and an immunity against the poisons or the toxins of bacteria, that is, a *toxin immunity*. The first is often called an *anti-infectious immunity* in the sense that the protective substances formed by the immunized body are directed against the infectious agent itself. These may give rise to the destruction of the representative infectious agent already localized in the tissues or may prevent the growth and spread of those subsequently introduced, so that they die or are eliminated. The second is often called an *antitoxic* immunity in the sense that the protective substances formed by the body enable it to neutralize the effects of the infectious agents already present; these are then destroyed by the alexins and phagocytes always found in the normal body. Bacteria robbed of toxicity in this way have the same relation to the body as saprophytes. Finally, we have a third kind of immunity in which the protective substances produced by one individual are transferred to another and confer upon this latter a protection like that in the original. This is called *passive immunity*, and may be produced by the transferal of serum of animals with anti-infectious or with antitoxic immunity. In passive immunity the individual receiving the immune cerum merely absorbs this serum and manufactures no protective substances, while in active immunity, either anti-infectious or antitoxic, the immunized individual makes its own protective substances either in the cells of the spleen or bone-marrow or in other cells, and gives them off to the blood.

In artificial immunization we are able to produce almost at will anti-infectious, antitoxic, or passive immunity, depending upon whether we employ the bodies of the organisms, their toxic products or the serum of animals already immunized. Thus in *diphtheria* we can produce an anti-infectious serum containing antibacterial substances, agglutinins, and bacteriotropins, but no antitoxins, by using the bodies of the diphtheria bacillus washed free of toxins. Or we may employ the toxins alone in immunization and get an antitoxic immunity. Finally we may transfer either type of immune serum and get passive immunity with our anti-infectious or anti-toxic serum. The same holds true of infections with *Bacillus pyocyaneus* where we may employ both the bacteria and their products and get a combined anti-infectious and antitoxic immunity (Wassermann[1]).

Any substance against which we can get an immune reaction in animals is now called an *antigen*. The antigens include bacterial toxins, the bodies of bacteria, various animal constituents like blood corpuscles and serum, animal cells, and plant proteins. The antigen is not necessarily poisonous in the sense that it always causes the death of the injected animal. It must however, combine in some way with the cells of the body which then responds by the production of a substance having a specific reaction for the material injected. When we use the bodies of the bacteria we get agglutinins and bacteriolysins, bacterial toxins give rise to antitoxins, blood-corpuscles to hemagglutinins and hemolysins, soluble proteins to precipitins and complement-fixing substances. The recent studies on the many types of substances

which may act as antigens and on the various immune bodies, and the demonstration that practially all the immune bodies follow the same law of production in the animal body and of combination with their specific antigens, have led to a belief that we may be dealing with a single immune substance in artificial immunization, its mode of action being dependent upon the physical properties of the antigen. That is, when we employ formed element, like bacteria and blood-cells we get agglutinins and bacteriolysins. Whereas when we employ soluble poisonous substances like toxins we get antitoxins. The question is by no means settled whether we have only one immune body capable of reacting in various ways or a multiplicity of immune bodies. It must always be remembered that the immune reaction is a *specific reaction* whether we have a single immune substance or a multiplicity of substances; that is, the immune materials produced by the injection of the diphtheria bacillus or its products are always specific for this organism.

Considerable discussion has taken place as to the chemical nature of the substances which can act as antigens. The majority of antigens of both animal and plant origin give protein reactions and Ehrlich's original view was that only protein materials cause the elaboration of antibodies in animals.[2] Split proteins, and protein decomposition products do not have this capacity. Interesting exceptions to this general rule have been noted however. Thus it has been shown by Abel and Ford[3] that the blood dissolving constituent of *Amanita phalloides*, against which an antihemolysin can be made by the immunization of animals, is not a protein, but a glucoside. The importance of carbohydrates in immunity reactions has been recently emphasized by Avery and his co-workers[4] who have found that the poisonous substance of certain pneumococci consists of two constituents, one a protein and the other a carbohydrate. In the case of the pneumococcus the carbohydrate by itself has no antigenic properties.

In addition to the antigens which cause the elaboration of specific products in animals it has been demonstrated by Forsman[5] and by Forsman and Widen[6] that certain tissues contain substances which cause the production of antibodies which are not strictly specific, but act on other tissues in addition to those used in the immunization. The organs of animals injected into rabbits give rise to antihemolysins for sheep blood-corpuscles. These are called *heterophile antigens* or Forsman's antigens. They are apparently attached to the lipoids of the tissues, especially to those soluble in alcohol and ether. Iijima[7] has found Forsman's antigens in certain bacteria like the dysentery bacillus.

## METHODS OF ARTIFICIAL IMMUNIZATION

Various methods have been devised by which we may artificially immunize animals and these may be classified as follows (Kolle[8]):

1. Active immunization with infectious agents alone with:
   - (*a*) Living infectious agents of full virulence.
   - (*b*) Living infectious agents of diminished virulence.
   - (*c*) Killed infectious agents as such or in chemical solution.
2. Active immunization combined with passive immunization, the immune serum being combined with:
   - (*a*) Living infectious agents of full virulence.
   - (*b*) Living infectious agents of weakened virulence.
   - (*c*) Killed infectious agents.
   - (*d*) Sensitized infectious agents.
3. Passive immunization with immune serum.

## PHENOMENA OBSERVED IN ARTIFICIAL IMMUNIZATION

Certain reactions are always observed in artificial immunization. These relate to the negative phase, the dosage, the site of inoculation, the height of the reaction and the length of protection.

**Negative Phase.**—It was formerly taught that a *negative phase* existed in the production of active immunity, before the resistance to the infectious agent was established; in this the resistance of the animal was less than normal. The negative phase was followed by a *positive phase* in which the resistance was increased. This phenomenon was explained by Ehrlich on the theory that the receptors of the infectious material combined with the amboceptors, no free amboceptors being left in the blood to protect the body. Wright[9] believed that the opsonins of the blood disappeared in the negative phase so that the bacteria were not phagocyted. Kolle states that the lessened content of the blood in bacteriolysins, agglutinins, antitoxins, and opsonins does not clearly indicate a period of lessened resistance on the part of the individual. For practical purposes the negative phase may be disregarded in immunization. It must always be remembered that in active immunization when large quantities of bacteria are introduced into the body they go to pieces and liberate endotoxins. The body may not have sufficient resistance against these endotoxins and die from their injurious effects.

**Dosage.**—In some instances the amount of the infectious material introduced is of moment and the immunity obtained may depend on the mass of material injected. In other cases the quantity of the infectious matter employed is of no consequence just so a reaction is produced. This is seen in cowpox vaccination where a small quantity of virus producing a limited local reaction causes just as long an immunity as multiple pustules (Kolle). Infectious agents of low virulence may produce just as good an immunity as those of full virulence and a light reaction from an agent of full virulence may last as long as a severe reaction from an agent of low virulence.

**Site of Inoculation.**—The place where the antigen is introduced may be of considerable importance in immunization. Thus Roux and Borrel[10] have shown that rabbits immunized by the subcutaneous introduction of tetanus toxin are not protected against intracerebral injection. Koch claimed that Pasteur's subcutaneous anthrax immunization was no protection against intestinal anthrax although it afforded a good protection against infection through the skin. In other instances immunity can be produced only when the antigen is introduced in a definite place in the body.

**Height of the Immunity.**—The immune substances produced by the body, like antitoxins, agglutinins, and bacteriolysins, are often measured to determine the degree of protection which the animal has come to possess against the infectious agent. With *antitoxins* our information is of considerable value since the presence of an antitoxin in the blood is a good measure of the protection of the individual against the infectious agent producing the specific toxin. This is seen in the Schick test in diphtheria where the presence of small quantities of diphtheria antitoxin in the blood-stream usually indicates that the individual has a resistance to infection by the diphtheria bacillus. *Agglutinins* cannot be used since their appearance does not run parallel with the appearance of immunity. *Agglutinins* for the typhoid bacillus may fail in a typhoid immune individual whose serum possesses specific bactericidal substances (Stern,[11] Kolle). Agglutinins really are an expression of the reaction to infection. Under certain conditions a high titre of agglutinins may give an indication of a high degree of

immunity, and in the immunization of animals with increasing doses of antigen the degree of agglutination is often a good index of immunity. Agglutinins are not applicable for the general determination of immunity. Powerful agglutinins may be present in the sera of moribund typhoid patients; in other diseases agglutinins may be present in sera which have little protective power, as in tuberculosis (Koch,[12] v. Behring,[13] Roemer[14]). *Bacteriolysins* are probably the most important index of immunity. In an individual recovering from typhoid fever or cholera the bacteriolysins which appear in convalescence are identical with the protective substances elaborated by man and animals in artificial immunization. Further, an agglutinating cholera or typhosus serum robbed of its bacteriolysins by chemical treatment has no protective properties.

**Complement fixation** has also been employed by many investigators to determine the height of the immunity. This reaction, known as the Bordet-Gengou phenomenon,[15] is more valuable for the differentiation of closely related organisms. It rests on the principle that complement is absorbed by a mixture of antigen and antibody, this being determined by a hemolytic system of blood-corpuscles serving as antigen and hemolytic antibody. The complement usually employed is guinea-pig serum titrated to the proper dilution. Various hemolytic systems may be employed, serum from rabbits immunized with guinea-pig corpuscles or sheep corpuscles, etc. For the diagnosis of bacterial infections the antigen is supplied by cultures of the bacteria, the antibody by the serum of man or infected animals. Cultures of bacteria may be used for the antigen or bacterial extracts. Besredka[16] finds that good antigens may be obtained by making salt solution suspensions, centrifugalizing, and adding about 90 mg. of common salt to 20 mg. of centrifugate. This is then thoroughly emulsified and distilled water added till the fluid is isotonic with the blood. The tubercle bacillus antigen of Wassermann and Bruck[17] is made by emulsifying the bacteria from about 10 agar slants in 10 c.c. distilled water. After shaking for twenty-four hours in a shaking machine 0.5 per cent. carbolic acid is added and the material then centrifugalized to clear it. The serum to be examined for the presence of antibody should be inactivated by heating to 57° C. one-half hour, then tested either before or after dilution.

The complement fixation test is employed especially in syphilis when it is known as the Wassermann reaction. Valuable results have also been obtained with it in the diagnosis of tuberculosis, gonorrhea, pertussis, and glanders. Somewhat less valuable results have been gotten in streptococcus infections, typhoid fever, and meningitis.

In tuberculosis the antigens may be made from the whole bacillus or the organisms may be dried and freed from fat by treatment with chemical reagents. In glanders the antigen may be obtained by Park's method.[18] The glanders bacillus is cultivated for twenty-four hours on salt-free veal agar with an acidity of 1.6 acid. The growth is emulsified in sterile distilled water, autolyzed at 80° C. for six to eight hours, filtered through paper pulp and Berkefeld candles and sterilized at 56° C. for one-half hour on three successive days. For other bacterial antigens the bacteria are usually cultivated on favorable media, washed off, emulsified in salt solution or in sterile water and subsequently made isotonic by the addition of salt.

**Length of Protection.**—The length of the protection against infectious agents depends upon a number of factors, the type of agent used, the kind of immunization, and the site of administration. In the inoculation with genuine smallpox virus the active immunity was apparently lifelong since the inoculated individual practically never developed smallpox although

exposed to it on countless occasions. In vaccination against smallpox with cowpox virus the immunity is supposed to last from seven to eight years. Accurate observations on this point are necessarily lacking but the immunity to the cowpox virus itself has been shown to last about this length of time. In vaccination with vaccines like those of cholera and typhoid the resistance to infection apparently lasts about two years.

In passive immunization the length of protection depends on two factors, the quantity of specific protective material employed and the method of administration. The protective substances are taken up from subcutaneous inoculation more rapidly than when administered intravenously and are eliminated more slowly. Immune materials from the same species give a more lasting immunity than that conferred by serum from different species. This has been shown, for instance, by v. Behring in horses immunized with tetanus antitoxin. Such immune bodies are called *iso-immune* bodies as contrasted with *hetero-immune* bodies derived from different species. The hetero-immune bodies are eliminated more quickly than the iso-immune bodies.

Passive immunity may be derived by inheritance from an actively immunized mother. The immune bodies may be carried to the body of the fetus "in utero" from the blood of the mother or absorbed through the milk. This has been shown especially by Ehrlich with ricin and abrin immunity.

## ACTIVE IMMUNIZATION WITH INFECTIOUS AGENTS OF FULL VIRULENCE

This type of immunization is seen in a few diseases of man and animals and depends in general on the principle that the introduction of a virus in a definite place like the skin, which is not its normal portal of entry, produces a different disease from the normal infection. In Asiatic cholera it has been shown by Ferran and Haffkine[19] that the subcutaneous introduction of the cholera vibrio is harmless, causing only a painful local reaction with fever. The organisms go to pieces in the subcutaneous tissues without producing an infection. Similar conditions are observed in cattle-plague (Lungenseuche). Rochelbryne (see Dieudonné) states that the natives of Senegambia immunize cattle by inoculating them on the skin under the tail with juice from the lungs of infected animals.

*Variolation* or the practice of inoculation against smallpox by the introduction of genuine smallpox virus is another example of this practice. Here the virus was often fully virulent and the disease produced was like the natural disease, but with a lowered mortality. Thus the mortality of natural smallpox varied from 60 to 70 per cent.; that of variola inoculata from 10 to 15 per cent. The disease was real smallpox, however. It was as infectious as the natural disease and could serve as a focus for epidemics with the usual mortality. The local lesions produced by the introduction of the infectious material beneath the skin might be insignificant; when the virus was introduced in the nose, as in the Chinese practice, extensive ulceration resulted. The diminution of the mortality in variola inoculata is usually attributed to the introduction of the virus in a region of the body which is not the normal portal of entry. In America, where inoculation was largely practised by Dr. Boylston, apparently an attenuated virus was developed which had the property of producing only a mild infection which conferred lasting protection.

The immunization of cattle against "Rinderpest" with the bile of infected animals is another example of this principle. It has been shown by Koch

that the bile contains the same infectious material as the blood, but has the remarkable property of making the infection localize in the subcutaneous tissues. Other examples are seen in sheeppox and in African fever.

Immunization by living infectious material of full virulence produces the highest degree of immunity known. Thus the resistance to smallpox which is developed in variola inoculata is practically identical with that following an attack of smallpox and is practically lifelong.

## ACTIVE IMMUNIZATION WITH INFECTIOUS MATERIAL OF LESSENED VIRULENCE

The virulence of the infectious material may be reduced by several procedures such as passage through different animals, treatment by chemical or physical agents, alone or in combination. The classic example of the use of a virus weakened by animal passage is that of vaccination against smallpox by cowpox virus. The immunity following vaccination is not lifelong as in variola inoculata, and lasts but seven to eight years. The nature of the cowpox virus has been much debated, but it has now been shown by Pfeiffer and Freyer[20] that genuine cowpox may be produced by the inoculation of cows with smallpox material. Cowpox is thus a true vaccine, that is, a smallpox virus so weakened by animal passage that it produces only a local reaction. A generalized vaccinia may develop and this may rarely be fatal (Dock[21]). This vaccinia is not smallpox and is not infectious. The change of the smallpox virus by passage through cows must be not merely a weakening of the virus, but a profound alteration in its properties as a result of which a different disease is produced by it.

The weakening of a virus by animal passage was practiced by Pasteur[22] in swine erysipelas. It was shown by Pasteur that the organisms of swine erysipelas when passed through rabbits, were weakened for swine; when passed through pigeons were increased in virulence for swine. Swine were first inoculated by the rabbit vaccine (Vaccine I) and then in twelve days by the pigeon vaccine (Vaccine II). Excellent results were sometimes obtained but the weakening of the virulence was not constant and there is a great difference in the resistance of different breeds of swine to the natural disease.

Recently the practice of immunizing cattle against bovine tuberculosis by the use of human tubercle bacilli which have a low virulence for cattle has been advocated by Koch and von Behring, but the results are still open to discussion.

The weakening of a virus by chemical methods is seldom practiced except in anthrax. Here the addition of certain chemicals to the culture media in low concentration and the prolonged cultivation of the organism in these media produces a strain which has lost its property of forming spores and has a lessened virulence. Such a strain may be used as a vaccine against the disease.

The physical methods to diminish virulence and produce viruses of less strength are chiefly *heat* and *drying*. Vaccines were first made by heating in anthrax and in symptomatic anthrax (Rauschbrand) by Pasteur, Roux, and Chamberland.[23] Virulent strains were cultivated at 39° to 40° C. and a regular loss in virulence was found to occur from day to day. Diminution in virulence from drying is best exemplified in rabies inoculation. Here the normal virus found in the street dog suffering from hydrophobia (street virus) was first converted into a virus of constant strength (fixed virus) by passage through rabbits by subdural inoculation. The spinal cord of the rabbits was now taken out and dried over caustic potash at 22° C. and the

strength of the virus found to depend upon the length of time during which it was dried. The longer the cord is dried, the weaker becomes the virus.

Recent investigations by Höyges[24] suggest that in this drying the organisms responsible for rabies are gradually killed off. The virus may not be weakened, but lessened in amount.

## IMMUNIZATION WITH KILLED INFECTIOUS AGENTS

The ability of *killed cultures* of micro-organisms to confer immunity was first proved experimentally on animals by Pfeiffer[25] and by Brieger, Kitasato, and Wassermann.[26] Cultures of the typhoid bacillus and the cholera vibrio were killed by heat and found to produce an immunity against infection by these organisms. Killed cultures were apparently first employed for man by Kolle, who used them in an attempt to protect against typhoid fever. The grade of immunity was determined by the amount of bacteriolysins in the blood of the inoculated. This type of vaccination was first tried out on a large scale by Wright, who vaccinated the British Troops in South Africa against enteric fever. In America it was developed chiefly by the work of Russell.

The organisms used for vaccination may be killed by heat or by the addition of chemicals.

It is a general rule that an immunity against the infectious agent requires the use of the intracellular material of the bacterial bodies which has the capacity to act as an *antigen* and call forth the production of immune substances in the inoculated. These intracellular substances are best released by heat. They may also be released by the pressure of 400 to 450 atmospheres (Buchner, Macfadyen, and Hahn).

Practical results of value have been obtained by the use of killed cultures chiefly in cholera, plague, and typhoid fever. According to Kolle the protection in cholera from killed cultures is the same as from the living organism introduced subcutaneously. This is due to the fact that in man living organisms put beneath the skin do not increase, but disintegrate and give up their intracellular substances. The same thing probably holds true for the typhoid bacillus, but observations on this point are lacking.

Killed cultures in general do not give rise to such profound immunity as living organisms either of full or low virulence. To produce approximately the same effect more of the killed infectious agent is necessary or repeated dosage must be resorted to. Even then the immunity is not so lasting. This has been shown experimentally in plague where a complete protection is afforded in guinea-pigs only by living organisms (Kolle and Otto[27]).

In man it is too dangerous to use living bacteria, and killed cultures must always be employed in vaccination.

With some organisms *polyvalent* vaccines may be used, that is, different strains combined in various proportions; each strain calling forth a different immune response in the individuals. In cholera it has been shown, however, that the immunizing property of the cholera vibrio is the same for all strains. This probably holds true of plague.

Vaccination may be carried out against a particular disease by vaccines prepared from the infectious agent of this disease. Or vaccines from different organisms may be combined and administered in the hope of affording protection against a number of diseases at the same time. Thus vaccines have been made from typhoid, paratyphoid, and dysentery bacilli, and the cholera vibrio. Such vaccines are often called polyvalent vaccines. They are, in reality, *multiple* or *combined* vaccines.

## METHODS OF PREPARING VACCINES

In the preparation of vaccines for therapeutic immunization the bacteria are usually grown on suitable media, washed off in sterile salt solution, and killed by heating to 57° C. half an hour. The most important and difficult step in the procedure is their standardization, *i. e.*, the determination of the number of bacteria in the emulsions and the amount of the material suitable for dosage. Various methods for this have been suggested. In the work of Wright and Douglas[28] the number of bacteria was determined by mixing the organisms with blood. The number of bacteria and the number of corpuscles is counted in the field of the microscope. From the ratio between the two the number of bacteria in the emulsion may be determined since normal blood always has a constant composition. The bacteria may also be counted directly by means of a hemocytometer, as first suggested by Mallory and Wright.[29] The suspension is diluted with salt solution or some dilute anilin dye like alcoholic methylene-blue. Measured quantities are now placed in the chamber of a Thoma-Zeiss hemocytometer which has a uniform depth of 0.02 mm. The number of bacteria may easily be counted in the requisite number of fields by using a high dry lens or an oil immersion lens, and from this figure the number of bacteria in the original suspension may be calculated. Finally the bacteria may be centrifugalized in graduated tubes as first proposed by Hopkins.[30] These tubes have a capacity of 10 to 15 c.c., have a graduated tip about 1 inch in length. This tip has a capacity of about 0.05 c.c. graduated in 0.01 c.c. quantities. The bacterial suspensions are first filtered through sterile cotton to remove foreign particles, then centrifugalized for half an hour at about 2800 revolutions per minute. After removing the supernatant fluid and the bacteria down to the 0.5 c.c. mark the sediment is pipetted off, taken up to 5 c.c. sterile salt solution giving a 1 per cent. suspension. Zinsser[31] states that 0.05 c.c. of streptococcus sediment obtained in this way represents 16 mm. of dried bacterial substance. The number of organisms per cubic centimeter in a 1 per cent. suspension varies from 2,500,000,000 of encapsulated pneumococci to 10,000,000,000 of *Staphylococcus aureus* and *albus*.

After standardization of the vaccines they are preserved in salt solution to which 0.5 per cent. carbolic acid is added. Dilutions are now made so that each cubic centimeter of material contains 100,000,000 to 500,000,000,000 of organisms.

Vaccines can also be standarized by the use of a nephelometer, as pointed out recently by Dunham.[32] Here the turbidity of the vaccines is compared with standards of turbidity corresponding to known bacterial suspensions and the number of organisms in the vaccines thus determined.

For specific vaccine therapy autogenous vaccines are now generally employed, *i. e.*, vaccines made from strains of organisms obtained from the patient himself. For prophylactic immunization against typhoid fever, dysentery, and cholera selected strains are employed in which low toxicity is combined with high protective value. In America typhoid vaccination is usually carried out with some well-known strain of the typhoid bacillus such as the Rawlings strain obtained by Russell from England. This is mixed with Paratyphoid A and B, so that each cubic centimeter contains 1,000,000,000 typhoid bacilli, 750,000,000 of each paratyphoid organism. Doses of 0.5 c.c., 1 c.c., and 1 c.c. are given seven to ten days apart.

Other vaccines which have been used with more or less success are the sensitized vaccines of Besredka[33] in which the organisms are treated with specific immune serum and lipovaccines in which the bacteria are dried at 53° C. for eighteen to twenty-four hours, ground up in a grinding machine

for periods varying from four to twenty-four hours, then mixed with 2 parts of cottonseed oil (Wesson's) and 1 part of lanolin and again ground for twelve to sixteen hours. Oil is now added to bring the mass up to the desired volume and sufficient chlorbutinol to give a 0.5 per cent. suspension. This is now ground again for four to twelve hours. The final volume is adjusted to give a lanolin content of 2 per cent. The content of bacteria in the final product is standardized by weight, the requisite dosage being published by the Army Medical School.

In this country favorable results from the use of Besredka's sensitized vaccines have been reported by Gay and Claypole.[34] Lipovaccines have not come into general use.

## PASSIVE IMMUNIZATION

In passive immunization the individual receives the serum containing protective substances manufactured by others. The treatment of patients with diphtheria antitoxin as a *curative* measure is an example of passive antitoxic immunization by diphtheria antitoxin obtained by the active immunization of horses. Here the antitoxin neutralizes the poisons of the diphtheria bacillus which is already implanted in the body and elaborating its toxic products. Passive immunization may also be carried out by diphtheria antitoxin as a measure of *prevention*, the antitoxin being administered to individuals who have no antitoxin of their own, as shown by the Schick test and who are believed to be susceptible to infection by the Klebs-Loeffler bacillus. In this type of passive immunization the neutralization of the poisons of the organism as soon as it invades the body and begins to secrete them evidently prevents further growth of the organism and renders it a harmless saprophyte. In tetanus the administration of the antitoxin has little or no effect when the disease is once established, since the toxins have already become attached to the nerve tissues where they cannot be reached by the antitoxins. The antitoxin may be administered as a *preventive* remedy. Its presence in the body of individuals infected by the tetanus bacillus neutralizes the tetanus toxin as it is produced and before it combines with the nerve centers. The tetanus bacillus thus robbed of its toxin also becomes a harmless saprophyte.

Antiendotoxic sera may be employed in passive immunization as with typhoid and cholera. Here the results are not as favorable and a liberation of endotoxins which cannot be neutralized is often suggested as an explanation.

In all types of passive immunization the body merely receives the protective substances and its work is limited to absorption as contrasted with active immunization where the body must work and manufacture its own protective substances.

## ACTIVE AND PASSIVE IMMUNIZATION COMBINED

Several combinations of the infectious agent with protective sera have been employed. Lorenz[35] has combined virulent infectious agents with high powered sera in swine-erysipelas. The animals are first given serum; then three to five days later virulent cultures. In such combinations generalized reactions do not occur and a lasting immunity develops. In "Rinderpest" a high grade serum accompanied by a virulent "Rinderpest" virus has been employed by Sobernheim.[36] The animals develop a mild attack from which they recover and develop a high degree of immunity. Combined immunization has also been employed in foot-and-mouth disease, anthrax, and occasionally in plague as by Kolle and Otto in guinea-pigs. In America

combined immunization is followed chiefly in hog-cholera where immune serum and virulent hog-cholera virus are sometimes administered simultaneously.

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## Chapter VIII

# THE ANTITOXINS

### GENERAL CONSIDERATIONS

*Antitoxic immunity* or a resistance to the action of poisons or toxins like those of diphtheria and tetanus, as well as certain other poisons of animal or plant origin, is due to the presence in an individual of protective substances, antitoxins, which neutralize these poisons. It is distinguished from *anti-infectious immunity* in which the infectious organisms are themselves destroyed or made harmless by the protective substances. This type of resistance may be *active*, the individual manufacturing his own antitoxins, or *passive*, the individual receiving antitoxins from others actively immunized. As already stated it is said that Mithridates was in the habit of immunizing himself against poisons by eating the blood of ducks fed on them and the term Mithridatism signifies an artificial antitoxic immunity. It is also claimed that the snake charmers in India accustom themselves to the bites of snakes by voluntarily submitting to the bites of less deadly species. Modern knowledge of antitoxic immunity began with the discovery of v. Behring and Kitasato[1] in 1890 that animals could be made resistant to the poisons of the tetanus bacilli by treatment with small doses and that this resistance depended on protective substances circulating in the blood-stream which neutralized the action of the tetanus toxin. These protective substances were later called *antitoxins*. Subsequently von Behring and Wernicke[2] demonstrated specific antitoxic substances in the blood-serum of animals immunized to diphtheria. Other species of bacteria were almost immediately investigated to determine whether they owed their pathogenic action to the elaboration of soluble poisons like the tetanus and diphtheria toxin and whether antitoxins could be obtained against them in animals, but it was demonstrated that only a small number of species act in this way. Toxins and antitoxins of the same type as tetanus and diphtheria toxin and antitoxin have been found only with *Bacillus botulinus*, the cause of meat poisoning, although the dysentery bacillus of Shiga apparently elaborates a similar toxic substance and an antitoxin can be obtained against it in animals.

In addition to bacteria and their poisonous products, which have been investigated most thoroughly for their ability to cause the production of antibodies in animals, many other poisons have been studied along the same lines. Antitoxins against the venoms of poisonous snakes have been obtained by Phisalix and Bertrand,[3] Calmette[4] and by Flexner and Noguchi.[5] Ehrlich[6] has shown that animals can be immunized against plant poisons like ricin and abrin and this immunity also depends on the presence of specific antitoxins, antiricin and antiabrin. It has also been pointed out by Ford[7] that the hemolytic constituent of poisonous mushrooms (*Amanita phalloides*) acts like a true toxin, the administration in small doses to animals causing the elaboration of specific antihemolysins.

The true or soluble toxins, as they are sometimes called, are elaborated by only a few species of bacteria, but have been studied with great care

because of the immense clinical importance of their antitoxins. Various criteria have been suggested by means of which soluble toxins can be defined. The most important of these are their heat-lability, their action in small doses after a definite latent period or time of incubation, and their specificity. Their chemical composition has not been determined. The prime characteristic of a toxin, however, lies in its ability to call forth the production of an antitoxin in animals. To produce antitoxins the toxins must be *soluble* and present in culture media in fairly considerable quantity. *Anti-endotoxic sera* can also be produced in animals which will neutralize the *endotoxins* of pathogenic bacteria up to a certain point. These antiendotoxic sera differ from true antitoxins in several particulars. They fail to neutralize the endotoxins according to the law of multiples which is usually followed in the neutralization of soluble toxins by their antitoxins. The principal difference lies in the fact that complement is necessary in the union of antiendotoxic sera and endotoxins. It has been shown by Pfeiffer and Bessau[8] that complement fixation always occurs when typhoid or cholera serum is mixed with typhoid or cholera endotoxin. This does not occur in the union of a true toxin and antitoxin as has been shown for tetanus by Wassermann[9] and Bruck[10] and Neufeld and Dold.[11] With antiendotoxic sera the action is analogous to the action of amboceptor and complement. In this reaction the molecules in the bacterial cell are altered chemically, the amboceptors being produced by the animal in response to the injection of integral parts of the bacterial bodies. The true antitoxins are due to the injection of secretory products of the living cells. As soon as specific soluble toxins are obtained from organisms like the typhoid bacillus and the cholera vibrio we should, of course, be able to obtain true antitoxins since the production of antitoxins is the principal criterion in our definition of a true soluble toxin.

## METHODS OF ANTITOXIN PRODUCTION IN ANIMALS

In the practical production of antitoxic sera it is first necessary to obtain good soluble toxins. These may be secured by cultivating the organism in particular media, as with diphtheria and tetanus, or by the use of strains of the organisms which are good toxin producers, as in Park's diphtheria bacillus No. 8. The toxic solution should be carefully standardized on animals, and the first doses given should be of such a character as not to affect the animal too much and yet give rise to a reaction. Sometimes the unaltered toxin is used, but in general it is somewhat weakened by heating to 60° C., or modified by chemical treatment, as by means of trichlorid of iodin or Lugol's solution. Frequently the first few doses are composed of toxin neutralized by antitoxin, after which small doses of toxin are employed. At the present time antitoxins are usually obtained from horses after subcutaneous administration of toxins. The animals show a severe local effect, consisting of a painful infiltration of the tissues at the site of injection and a general febrile reaction. The reaction with diphtheria is generally greater than with tetanus. The antitoxins appear in the blood-serum and lymph and are also present in the cell-free edema fluid, aqueous humor, saliva, urine, and lumbar fluid. Occasionally they are found in the milk.

The following protocol from Salomonsen and Madsen[12] gives the details of a typical immunization of a horse for the production of diphtheria antitoxin. The toxin employed was of medium strength, 0.1 c.c. killing a 500-gram guinea-pig in forty-eight hours.

IMMUNIZATION OF HORSE FOR DIPHTHERIA ANTITOXIN PRODUCTION

*Salomonsen and Madsen*

Gravid Mare, Weight 665 Kg.

| Day. | Toxin dose. | Day. | Toxin dose. | Remarks. |
|---|---|---|---|---|
| | c.c. | | c.c. | |
| 1 | 1 | 104 | 800 | |
| 6 | 1 | 119 | 1000 | |
| 12 | 3 | 135 | .... | Bleeding from vein. Antitoxic serum has value of 150 I. E. |
| 15 | 5 | 154 | .... | Birth of a foal. |
| 23 | 10 | 177 | .... | Bleeding from vein. Antitoxic serum has value of 45 I. E. |
| 27 | 20 | 184 | 100 | |
| 36 | 25 | 188 | 200 | |
| 41 | 50 | 195 | 400 | |
| 45 | 75 | 205 | 700 | |
| 50 | 100 | 213 | 800 | |
| 57 | 150 | 223 | 600 | |
| 72 | 250 | 232 | 600 | |
| 81 | 450 | 242 | 1000 | |
| 92 | 600 | 252 | .... | Bleeding from vein. Antitoxic serum has value of 120 I. E. |

## SOURCE OF ANTITOXIN IN THE BODY

The antitoxins are specific products of the cells of the body which react to the stimulation by the toxin, but there is still a good deal of uncertainty as to which particular cells take part in this antitoxin production. Wassermann[13] believes that every cell injured by the toxin is a potential producer of the antitoxin. The explanation suggested by Ehrlich for the formation of antitoxin is now usually accepted, at least as a working hypothesis. According to Ehrlich the majority of poisons like the narcotics and alkaloids do not form a firm union with the cells of the body, but are either digested unaltered or act by lipoid solubility, chemical synthetic processes playing no special rôle in the reaction. Other poisons of which the soluble bacterial toxins are the outstanding examples are *assimilative*, that is, are taken into the cells and become part of the cell protoplasm. Thus an alkaloid like strychnin acts like saccharin which is not assimilated, while a true toxin acts like sugar which is assimilated. Ehrlich now designates certain molecular groups in the substance which can be assimilated and combine with the cells as *haptophoric groups*. The cells themselves have corresponding groups which can anchor the assimilated materials, and these Ehrlich calls *receptors*. In addition to the haptophoric groups of the assimilated material this material possesses other groups which vary according to the biological action of the substance. In toxins they are called *toxophor groups*, in ferments *zymophor groups*, in agglutinins *agglutinophor groups*. A toxin thus consists of two hypothetical groups of molecules, the haptophoric group by means of which it combines with the cells of the body, and the toxophoric groups by means of which it inflicts its specific injury on these cells. When the haptophoric groups combine with the receptors of the cells these receptors are first injured, giving rise to a defect in the cells. To compensate for this defect new receptors are manufactured by the cells and always in excess, in accordance with Weigert's law of compensation. The receptors thus produced pass into the blood, where they can also combine

with the haptophoric groups of the toxins. Since, however, the toxins act upon cells only by the combination of haptophoric groups with cell receptors, if these haptophoric groups are already united with free receptors in the blood, the toxins can no longer unite with the cells of the body which are thus protected from injury. The receptors of the cells produced in excess are discharged into the blood-stream and thus become the *antitoxins*. They are called haptines of the first order by Ehrlich. Furthermore, the functioning groups of material which can be assimilated are labile and easily destroyed, while the haptophoric groups are stable. A toxin may, therefore easily lose its toxophor group, only the haptophor group remaining. The toxoids arising in toxins as they are heated or as they deteriorate from age, which are still able to combine with antitoxins, are regarded by Ehrlich as haptophoric groups left behind after the toxophoric groups have been destroyed.

There are various objections to the complete acceptance of Ehrlich's theories as to the origin of antitoxin. The chief discussion centers about the hypothesis that the cells which are injured are themselves producers of antitoxin. It has been demonstrated by Wassermann and Takaki[14] that the brain substance of guinea-pigs which is injured in tetanus intoxication is capable of combining with and neutralizing tetanus toxin. Ranson[15] has also shown that in animals which can produce tetanus antitoxin the toxin disappears from the blood and forms a true combination with the organs, being neutralized in the central nervous system. It has not yet been proved that the central nervous system with which the tetanus toxin combines is definitely the site of origin of the antitoxin.

Metchnikoff[16] locates antitoxin production chiefly in the *macrophages*. Parts of the body, like the bone-marrow and spleen, which are rich in macrophages, are not more laden with antitoxin than other organs of the body, however.

Gay and Clark[17] have found that antibody formation can be prevented in animals by saturation with trypan-blue, a dye which is known to be selectively stored by the reticulo-endothelial system of Aschoff. This seems to be due to filling the cells with the dye stuff. As a result hemolysins and precipitins are not formed.

## ACTION OF ANTITOXIN ON TOXIN

When an animal is given a fatal dose of bacterial toxins the administration of antitoxin will protect it and prevent death from the intoxication. In general, the antitoxin must be administrated before the toxin has had an opportunity to combine with the cells and inflict its injury. Recent experiments indicate that antitoxin can unite only with free toxin; the toxin already in combination with the cells cannot be neutralized by antitoxin even if the latter be given in excess. Occasionally cells injured by toxin seem to regenerate after the administration of antitoxin, and in such cases the toxin in the cell may have been neutralized by the antitoxin (Wassermann and Wassermann).

Antitoxin can combine with toxin "in vitro," making it non-toxic. There is, however, no destruction of toxin. It has been shown by Calmette in mixtures of cobra venom and antitoxin that the antitoxin may be destroyed by heat, leaving behind the active venom. It has been found by Wassermann that in *Bacillus pyocyaneus* toxin-antitoxin mixtures the heat-resistant *pyocyaneus* toxin is liberated by heating these mixtures. Morgenroth has shown that in year-old mixtures of toxin and antitoxin destruction of toxin does not take place. By treatment of the mixtures with dilute HCl both the toxin and antitoxin may be obtained. Ehrlich is of the opinion that

there is a *chemical combination* between the toxin and antitoxin. Ehrlich and Knorr[18] have found that the union takes place according to laws which hold for chemical reactions, the concentration, temperature, and salt content all affecting the result. The avidity of toxin for antitoxin is not always the same, but varies with the kind of toxin. The union of tetanus toxin and its antitoxin takes place more slowly than the union of diphtheria toxin and antitoxin, or snake venom and antivenom. It has been found by Martin and Cherry,[19] for instance, with a neutral mixture of snake venom and its antitoxin, that after filtration through gelatin filters the filtrate again becomes toxic. This is explained on the theory that the gelatin lets the smaller colloid molecules of toxin through, but not the larger molecules of antitoxin. After a certain time the combination becomes so firm that a separation of the toxin from the antitoxin by filtration is not possible.

The combination of toxin and antitoxin has been investigated in diphtheria with especial labor by Ehrlich and his collaborators. According to Ehrlich's original theory diphtheria toxin and antitoxin combine according to the laws of simple multiples, that is, 10 units of antitoxin neutralize 10 units of toxin, 100 units of antitoxin neutralize 100 units of toxin, 1000 units of antitoxin neutralize 1000 units of toxin, etc. The phenomena observed in the study of toxins of different sources and ages, and the rates in which these are neutralized by antitoxin, revealed that the law of multiple proportions as shown in simple chemical reactions is not strictly followed. This is expressly true when toxin is added to antitoxin in fractions, since a combination of toxin and antitoxin may be *neutral* when the two ingredients are added in their entirety at the same time, but *toxic* when the toxin is added to the antitoxin little by little. Furthermore, different observers may obtain quite different values for the same lot of antitoxin, using as a unit that amount which will neutralize 100 minimum fatal doses of toxin, but employing toxins of different sources for the standardization of the antitoxin. Different results were also obtained from time to time with the same sample of toxin and antitoxin, suggesting that some kind of change had taken place in one or both of the ingredients. A freshly prepared toxin gave in general a higher antitoxin unit than an old toxin, that is, fresh toxin is neutralized by a smaller amount of antitoxin than is old toxin.

The combination of diphtheria toxin and antitoxin was now subjected to a searching investigation by Ehrlich from a variety of angles. In his studies he employed certain amounts of toxin and antitoxin which are now used almost universally. Thus that amount of diphtheria toxin which just kills a guinea-pig of 250 grams weight on the fourth or fifth day is called the mimimum lethal dose (M. L. D.). A unit of antitoxin is that quantity which exactly neutralizes 100 M. L. D. Since, as already stated, different observers might obtain different values for the antitoxin, depending upon the toxins they employed, Ehrlich started his observations with a carefully standardized antitoxin and tested various toxins against this. The amount of toxin which was exactly neutralized by one unit of this standard antitoxin he called Limes nul (Lo); the amount of toxin which can be mixed with a unit of antitoxin and still cause the death of a guinea-pig on the fourth or fifth day was designated Limes plus (L+). Theoretically the Lo dose of toxin should always be equal to 100 M. L. D. and the L+ dose equal to 101 M. L. D., since it represents the 100 M. L. D. neutralized by the unit of antitoxin, and, in addition, one fatal dose or just that amount of toxin which will kill a guinea-pig on the fourth or fifth day. Practically these results were seldom or never obtained when the mixtures were tested on animals, and the relationships of the toxin and the antitoxin varied markedly with the

samples of toxin examined and with their age. For example, a sample of toxin might give an M. L. D. of .0025 c.c. Theoretically the Lo dose should be 100 × .0025, which equals 0.25 c.c., and yet it might be only 0.125 c.c. Again, the L+ dose should be theoretically 0.2525 c.c., and yet it might be only 0.25 c.c. Furthermore, as toxin aged and deteriorated larger and larger amounts were required to kill guinea-pigs, that is, the toxic solutions were less potent and the M. L. D. correspondingly lower or of greater bulk. In such old toxins the Lo value remained the same, indicating that the deteriorated toxin had practically the same combining value for antitoxin as the fresh toxin. Ehrlich now studied the combinations of toxin and antitoxin by the methods known as "partial absorption." Here the same Limes + dose of toxin is combined with different amounts of antitoxin. The results obtained by this method also failed to fit in with theoretical considerations as shown by the following table taken from Park and Williams (p. 179):

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Antitoxin | 0 unit | + | toxin L | + | dose | = | 85.0 M. L. D. |
| | 0.25 " | + | " | + | " | = | 60.0 M. L. D. |
| | 0.80 " | + | " | + | " | = | 10.0 M. L. D. |
| | 0.90 " | + | " | + | " | = | 3.5 M. L. D. |
| | 1.00 " | + | " | + | " | = | 1.0 M. L. D. |

Here the L+ dose contains only 85 M. L. D. when it should theoretically equal 101 M. L. D., while the values obtained for the neutralization of the toxin by different amounts of antitoxin do not strictly follow the law of multiple proportions.

Diphtheria toxin can also be standardized on guinea-pigs by the intracutaneous method. That amount which just causes a necrosis of the epithelium is known as Limes necrosis (L. N.). It usually equals 1 to 500 M. L. D.

To explain the results obtained by this method of partial absorption as well as the phenomena observed in the neutralization of old deteriorated toxins as compared with fresh toxins Ehrlich assumed that the diphtheria bacillus produced two primary substances, a *toxin* and a *toxone*. The acute death of the inoculated animals was attributed by Ehrlich to the action of the toxin, the subcutaneous edema and the late paralysis to the toxone. Both toxin and toxone were capable of combining with and binding antitoxin, but the toxin was supposed by Ehrlich to have a weaker affinity. In the deterioration of diphtheria toxin to less active poisons, the toxin was supposed to change to *toxoid* which might also combine with diphtheria antitoxin, but have a different combining affinity. The toxones might also deteriorate to toxoids (sometimes called toxonoids). Finally, Ehrlich came to the conclusion that the combining value of an antitoxic unit was 200, not 100; that is, 1 antitoxic unit represented 200 valencies, and this amount when mixed with an L+ dose left only 1 unit of toxin free. Theoretically, therefore, in diphtheria "*toxine*" which contained only *toxin*, that is, poisonous constituents not yet deteriorated, the Lo dose would contain 200 M. L. D. and the L+ dose 201 M. L. D. Since toxin and toxone deteriorate rapidly, these theoretical values would be found with only unchanged toxin and toxone in freshly prepared "toxines." Practically the highest value ever obtained in fresh diphtheria "toxine" is one by Madsen, where the Limes nul equalled 160 M. L. D. The composition of diphtheria toxin as hypothecated by Ehrlich has met many objections. The distinction between the ingredient *toxin*, which is responsible for the acute death of guinea-pigs, and the *toxone*, which causes the edema and paralysis, is by no means always accepted; there is a good deal of evidence that the lesions in animals are all due to a single

substance. When large amounts of the diphtheria poison are used, the animals die acutely from the effect of the poison on the nerve centers and the internal organs. When small amounts are used, this effect is not seen and the animals have time to respond to the local injection by the production of the subcutaneous edema. The paralysis may be due to the same poisonous constituent and appear only in animals which survive for a long period. It may be emphasized in passing that this variation in the lesions produced by diphtheria toxin with the amount administered to the animals and the length of survival is a phenomenon seen with other poisonous substances. Arrhenius and Madsen[20] claim that toxin and antitoxin combine chemically, but according to the Guldberg-Waage's law of *mass action*, and do not accept Ehrlich's views as to toxoid. The law of mass action applies only to reactions which are fully reversible and the toxin-antitoxin combination has not thus far been shown to be fully reversible. Thus Danysz[21] has found with mixtures of ricin and antiricin that the neutralization takes place only when the entire quantity of antitoxin necessary for neutralization is added at once (Danysz phenomenon). This is explained by Arrhenius on the basis of the chemical laws seen in the neutralization of monochloracetic acid with sodium hydrate.

Other objections have been raised to Ehrlich's theories as to the chemical union between toxin and antitoxin, and the subject is by no means clear. Bordet[22] believes that the combination of toxin and antitoxin is a kind of mechanical adaptation of colloids due to molecular adhesions. The toxin-antitoxin combination takes place according to laws which are somewhat different from the laws of mechanical absorption, chiefly in that it is not strictly a reversible reaction.

Another method for determining the value of antitoxic sera has recently been devised by Ramon,[23] who has shown that mixtures of diphtheria toxin and antitoxin give a visible flocculation or precipitate after standing several hours at room temperature. This is known as Ramon's flocculation test, It is a specific reaction and does not occur with normal sera, other antitoxins, or with antitoxins heated to 100° C.

In carrying out the flocculation test the amount of toxin in any particular toxic filtrate is determined by titrating it by flocculation against one unit of antitoxin standardized by the Ehrlich method. This amount of toxin is known as the L f dose. The amount of diphtheria antitoxin in serum which causes a flocculation with known quantities of toxin can now be determined and the antitoxic value of any particular serum can be obtained. These values correlate closely with the values obtained by therapeutic tests on animals (Ramon, Scholz[24]). Scholz finds that the flocculation test can also be used to determine the value of tetanus antitoxin.

In very careful work the mixtures of toxin and antitoxin should be kept at 37° C. (Glenny and Okell[25]).

## CHEMICAL NATURE OF ANTITOXINS

It is generally believed that antitoxins are proteins and their chemical reactions suggest this strongly. They are easily injured by heat and are destroyed at 60° to 70° C. in a short time. They are sensitive to pepsin-hydrochloric acid and resist tryptic digestion. Antitoxic sera give, of course, all the usual protein reactions. The antitoxins may be precipitated from the sera by reagents which throw down the globulins, corresponding to pseudoglobulins in horses, to euglobulins in goats. When dry, antitoxins resist temperatures of 110° to 140° C. for a short time. They are injured by direct light and oxygen and are destroyed by acids. Occasionally anti-

toxins may be kept intact for long periods. Thus tetanus antitoxin has been preserved by von Fedorow and Ikonnikow[26] for fifteen years, but this is very exceptional.

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## Chapter IX

# THE AGGLUTININS

The bacterial agglutinins were discovered by Gruber and Durham,[1] and our first comprehensive knowledge of their action came from the subsequent work of Durham,[2] who investigated the phenomenon in great detail. Gruber and Durham noted that if an immune serum be added to an opalescent mixture of bacteria the fluid clears and the homologous bacteria are deposited at the bottom as a coherent viscid mass while other bacteria are not so affected. By this agglutination the typhoid bacillus could be distinguished from the cholera vibrio. The application of agglutination as a diagnostic measure to determine whether patients were suffering from typhoid infection was first made by Widal,[3] although Greenbaum was already working on this phase of the problem in Gruber's laboratory. Since then the agglutination reaction, or the Widal reaction, as it is usually called, has proved of immense service in clinical investigation of the infectious diseases, especially in cholera, typhoid and paratyphoid fever, dysentery, and Malta fever.

The ability to cause the production of agglutinins in animals may be regarded as a constant feature of all pathogenic bacteria. The reaction was originally regarded by Gruber and Durham as an immunity phenomenon, while Widal considered it as essentially a reaction of infection. Non-pathogenic bacteria also may cause the production of agglutinins in animals. Durham has found them with *Bacillus megatherium*, Sternberg with Boas' lactic acid bacilli, and Vincent with *Bacillus mesentericus*.

Agglutinins are not only the product of bacterial invasion of the animal body. They arise on the introduction of red blood-corpuscles, spermatozoa, and certain of the protozoa like trypanosomes. At the same time many cells are agglutinated by materials other than normal or immune sera. Chemical substances like acids agglutinate bacteria, plant extracts like ricin, abrin, and the juice of *Amanita muscaria* agglutinate red blood-corpuscles. For the present we are considering only the bacterial agglutinins.

## THE PHENOMENON OF AGGLUTINATION

The reaction called agglutination consists in principle of a precipitation of bacteria in a fluid menstruum, broth, or salt solution, on the addition of a serum obtained from an animal to which the same bacteria have been administered. Coincidently the bacteria when motile lose their motility. If the agglutinated bacteria be broken up the organisms no longer form an homogeneous emulsion, but are bound together in small clumps which can be disintegrated with the greatest difficulty. The reaction may be observed in the test-tube by the *macroscopic* method, or in hanging drops of bacteria and serum under the microscope, the *microscopic* method. The reaction is essentially the same whatever method be employed to observe it, but in general the macroscopic method gives more reliable and accurate information.

The agglutinated bacteria are not markedly altered except for their loss of motility and stain the same way as before agglutination has taken place. Occasionally the organisms swell and assume capsular substances about them, but these changes are not essential parts of the reaction. Despite loss of motility the flagella are present and unaltered (Gruber,[4] Pfeiffer,[5] Joos[6]). The agglutinated bacteria are not killed in the reaction and often

retain full virulence. Killed bacteria give rise to agglutinins on introduction into animals, and both living and killed organisms are agglutinated by their homologous agglutinins. Smith and Reagh[7] and Orcutt[8] have pointed out that the bodies and the flagella of bacteria have different antigenic substances and produce different agglutinins on injection into animals.

The substance in the serum which takes part in the reaction is known as the *agglutinin*, while the substance in the bacterial cells which gives rise to the agglutinin on its introduction into animals is known as *agglutinogen*. The agglutinogen is identical with the agglutinable substance of the bacteria, which combines with the agglutinin in the agglutination reaction. The product of the agglutinogen and agglutinin is called the *agglutinat*.

Different bacteria agglutinate with different degrees of readiness, some easily, like *Bacillus typhosus*, *Bacillus coli*, the dysentery bacillus, the cholera vibrio, and certain types of *Proteus*. Others agglutinate with less ease, like the diphtheria and tetanus bacillus, tubercle, anthrax, streptococcus, and staphylococcus. Certain species are agglutinated with great difficulty, such as the encapsulated bacteria (rhinoscleroma and Friedländer). This resistance of encapsulated organisms to agglutination may be due to the presence of the capsular substance, since Jobling has shown that if an encapsulated rhinoscleroma bacillus be washed free from capsules it is readily agglutinated by an immune serum.

If bacteria be heated to 65° C. or treated with dilute acid at 37° C. they lose their agglutinability (Widal, Eisenberg and Volk[9]). Such heated inagglutinable bacteria are still able to combine with and bind agglutinin.

Pathogenic organisms when freshly isolated from the body are frequently inagglutinable by immune serum. The virulence of these organisms and their capacity to bind agglutinin is not lessened, as shown by Wassermann[10] and by Cole.[11] Inagglutinable strains can also be obtained by cultivation in immune serum as Cole has shown for *Bacillus typhosus*. Similar strains develop if the organisms are cultivated in colloidal sacs in the animal body, as Sacquépée[12] has found for *Bacillus typhosus* and McCrea[13] for *Bacillus enteritidis* of Gärtner.

## RELATION OF AGGLUTINATION TO IMMUNITY

The relation of the phenomenon of agglutination to immunity has been much discussed, but there is still some doubt in regard to it. The bacteria are not killed in agglutination; it is possible to immunize animals so that they have protective substances in their serum, but no agglutinins. Thus Widal and Nobécourt[14] have immunized mice with the urine of typhoid patients and have found that these animals resisted a fatal dose of the typhoid bacillus, but had no agglutinin for this organism. Brieger,[15] Schütze,[16] and Brieger and Mayer[17] have obtained strongly agglutinating sera in rabbits by treatment with a substance obtained from the typhoid bacillus. These sera showed no increase of bactericidal strength. Similar phenomena have been observed by Wassermann in animals immunized to *Bacillus pyocyaneus*. A further differentiation of agglutinins from protective substances has been obtained by Mayer[18] and by Neufeld,[19] who found that the granules of the cholera vibrio, freed from disolved products, caused the formation of cholera bacteriolysins in the animal body, but not of agglutinins. Paltauf[20] states that absolute proof has not yet been brought that agglutinins have nothing to do with the protection of the body from the invasion of pathogenic organisms, and it is still doubtful whether agglutination actually occurs in the body.

Agglutinins may be conveniently regarded as a third type of immune

substance in addition to bacteriolysins and antitoxins. They possess a strong affinity for the bacterial bodies and may be absorbed from sera containing them by treatment of the sera with the bacteria, as first shown by Castellani.[21] This is an *elective* absorption, since only homologous or related bacteria thus absorb agglutinins.

In the combination between the agglutinogen of the bacteria and the agglutinins of the serum, complement is not absorbed. Agglutinins are therefore somewhat like antitoxins which also unite with toxins without complement fixation.

## NORMAL AGGLUTININS

Agglutinins are present in normal sera for many types of bacteria, but they differ greatly in strength and variety. They may be found in man for *Bacillus coli*, but not for pathogenic organisms like plague and diphtheria. They are absent in the fetus and newborn and are higher in adults than in children. They are apparently related to the presence of the organism in the body. More *Bacillus coli* agglutinin is found in the blood of grown people than of the young. Normal agglutinins are thus an acquired property and may be due to the absorption of bacterial materials from the intestinal tract. Ribbert[22] has shown that bacteria are always in the mesenteric glands of animals and Ford[23] has found that bacteria are frequently present in organs like the liver and kidney, especially in full digestion. Micro-organisms are also demonstrable in the lymph during digestion as has been demonstrated by Rogosinski and by Nichols.

In addition to bacteria spores are frequently agglutinated by normal sera.

Normal agglutinins are *specific* and can be absorbed by bacteria from normal serum. Bordet has shown that the agglutinins in normal horse serum for the typhoid bacillus and the cholera vibrio can be absorbed by treatment of the serum with these organisms.

## IMMUNE AGGLUTININS

Immune agglutinins develop in the bodies of man and animals as a response to infection. They are found in typhoid fever in man for the typhoid bacillus and in Malta fever in goats for *Bacillus melitensis*. They may be produced experimentally in animals by the artificial introduction of organisms through various routes, the subcutaneous tissues, the veins, the stomach and intestines, and the lungs. Agglutinins develop in animals after the introduction of celloidin sacs containing organisms into the peritoneal cavity. Here the substances giving rise to the agglutinin, the agglutinogen, dialyze through the celloidin which acts as a semipermeable membrane.

Cold-blooded animals produce agglutinins for infectious agents even if they are not sensitive to infection by them. Thus Widal and Sicard have shown that frogs, turtles, and crocodiles can manufacture agglutinins for the typhoid bacillus.

Agglutinins can be produced both with living organisms and with killed cultures, the substance giving rise to the agglutinin (agglutinogen) not being destroyed by heat.

Immune agglutinins may be present in the blood of artificially immunized animals in large amounts. Van de Velde[24] has found an agglutination for *Bacillus typhosus* in a dilution of the immune serum of 1 : 1,000,000 and Durham one for *Bacillus coli* in a dilution of 1 : 2,000,000.

Agglutinins may persist in the body for long periods. Gruber has found them for seven to thirteen months in the blood-serum and peritoneal lymph after immunization. In typhoid fever agglutinins may last for years, usually however in moderate quantities.

Agglutinins are found in the blood and lymph, in the fluids of the serous cavities, in the milk, in the lacrimal secretion. They may pass from mother to fetus, but not regularly; do not arise locally after subcutaneous injection, but appear in the blood-stream; are not found in extracts of leukocytes.

Normal and immune agglutinins are generally regarded as identical substances. Both types are resistant to heat and cold and lie in approximately the same fraction of the serum containing them. Thus the normal agglutinin for *Bacillus typhosus* in the horse lies in the euglobulin fraction according to Eisenberg,[25] Landsteiner,[26] and Calcar,[27] while the immune agglutinin has been found by Pick[28] in the pseudoglobulin.

## SPECIFICITY

In general, immune agglutinins have a special affinity for the organisms used in the immunization of the animals producing them and react best with the same strain. The reaction cannot be regarded as absolutely specific and indeed it is by no means as specific as are other immune reactions like those with antitoxins and bacteriolysins. The agglutinins act most strongly on homologous bacteria, but act also on closely related organisms. We therefore speak of *principal* or *major* agglutinins (Haupt-agglutininen) which act on the specific organisms and *minor* or *partial* agglutinins (Mit-agglutininen) which act on other organisms that may be closely related, but need not be. Thus agglutinins for a member of the paratyphoid group are apt to act on other paratyphoids and even on the typhoid bacillus and on *Proteus*. According to Durham the agglutinogenic material of the bacteria is not a single substance, but made up of various components, a, b, and c, with a number of corresponding agglutinins in the serum A, B, and C. Certain agglutinogenic components of different types of bacteria may be the same. In consequence we have principal agglutinins for the strain used in immunization and partial agglutinins for bacteria which have some of the same kind of agglutinogen. The relations are very complicated, however, as shown by the recent exhaustive investigations of Krumwiede.

The production of partial agglutinins (Mit-agglutininen) depends somewhat on the animal which is used. Rabbit immune serum has as a rule less partial agglutinin than the immune sera obtained from horses or asses. The quantity of group agglutinin does not always determine the amount of partial agglutinin. It has furthermore been shown by absorption that when group agglutinins are taken out of an immune serum by treatment with homologous bacteria the partial agglutinins are also removed. Absorption of partial agglutinins, on the contrary, does not remove the group agglutinins.

In mixed infections homologous agglutinins may be present in the serum for all the infecting organisms. They also may be removed from the serum by absorption with homologous bacteria. In addition we sometimes have agglutinins in immune sera or in sera of diseased persons which have no capacity to combine with the organisms taking part in the immunization, but may unite with other organisms. These are called *heterologous* agglutinins (Neben-agglutininen). They are not the same as partial agglutinins. In typhus fever agglutinins are produced for strains of *Proteus vulgaris*. These are evidently heterologous agglutinins since the typhus patients are not infected by the strains of *Proteus*.

## COMPONENTS OF THE AGGLUTINATION REACTION

The two different substances concerned in *agglutination*, the *agglutinogen* and the *agglutinin*, and their product, the *agglutinate*, have been especially investigated and important facts determined in regard to them.

### THE AGGLUTINOGEN

Agglutinins arise in animals after immunization with living or dead bacteria and with germ-free filtrates. The filtrates of *Bacillus typhosus*, *Proteus vulgaris*, *Bacillus pyocyaneus*, and the cholera vibrio cause the production of agglutinins for these organisms when introduced into animals. The agglutinogenic substance must therefore be present in the bacteria and be given off to culture media. According to Paltauf it corresponds to the coagulable substance in the bodies of the bacteria. It has been shown by Nicolle,[29] however, that the agglutinogenic substance in old culture fluids is soluble in alcohol and ether, very resistant to high temperatures, is destroyed at 115° C. and resists tryptic digestion. The coagulable substance in culture filtrates is insoluble in alcohol and does not resist a temperature of 115° C. (Winterberg[30]). Pick has further shown that there are two coagulable substances in bacteria: one precipitated by alcohol and one soluble in this reagent. The first may be present in old broth cultures of *Bacillus typhosus* while the second is found in young *typhosus* cultures. The substance insoluble in alcohol is a protein split product. The alcohol-soluble substance is not protein, albumose, peptone, or nucleoprotein. Both these substances give precipitates in *Bacillus typhosus* immune serum. They may be the same as agglutinogens. Brieger and Mayer,[17] however, have obtained an agglutinogen from typhoid bacilli which is not a precipitinogen. Similarly Joos[6] has found a thermolabile agglutinogen in fresh cultures of *Bacillus typhosus*, destroyed at 62° C., as well as a thermostable agglutinogen. Buxton and Torrey[31] finally have found soluble agglutinogenic substances resisting 70° C. which produce two different agglutinins. There may be a number of different substances which are precipitated by immune serum and carry the bacteria down with them. Eisenberg and Volk[9] state that typhoid bacilli heated to 62° to 65° C. are no longer agglutinated by immune serum, but can combine with and bind the agglutinins in this serum. These authors differentiate in the agglutinable substance (or agglutinogen) of the bacteria a binding *thermostable* group which can stand 65° C. and a functioning *thermolabile* group destroyed at 65° C. The latter group actually precipitates the bacteria after the combination has been affected by the binding group. Agglutinogens in different bacteria do not have the same properties however. Thus Eisenberg and Volk[9] have shown that the cholera vibrio may be heated to 165° to 170° C. for half an hour and lose little of their agglutinability. Apparently therefore the agglutinable substance or the agglutinogen is carried by the bacterial protein or nucleoprotein and forms a specific combination with the agglutinins in the sera, the actual deposition of the bacteria being a secondary thing.

### THE AGGLUTININ

The agglutinating substance in the serum, or the agglutinin, is primarily a component of the protein constituents of the serum. According to Widal and Sicard the agglutinins are related to globulins, since reagents which precipitate globulins also throw down agglutinins. In cholera immune serum in horses the agglutinins lie in the euglobulin fraction. In the immune serum of goats, rabbits, and guinea-pigs the agglutinins also lie in the euglob-

ulin fraction. Agglutinins are, as a rule, easily destroyed by heat, but different types show different degrees of resistance. The agglutinins for tubercle and plague are destroyed at 56° C., while the cholera immune agglutinin in horses is destroyed at 65° C., but is more sensitive than the typhoid agglutinin (Pick[28]). Agglutinins resist digestion by pepsin, trypsin, and papayotin for twenty-four hours, but can be destroyed by long exposure to trypsin, in five days according to Pick. Joos believes that the agglutinin for *Bacillus typhosus* has two modifications, one destroyed or changed at 60° to 62° C. and one changed at 62° to 65° C. The *thermolabile* agglutinin is related to the thermolabile agglutinogen, the *thermostable* to the thermostable agglutinogen. Agglutinins may be so modified that they no longer agglutinate the bacteria, but still combine with them. These changed agglutinins are called agglutinoids.

### PROAGGLUTINOIDS

Occasionally agglutination fails to occur in concentrated immune serum when it takes place readily in dilutions of that serum. This is traced by Ehrlich to the presence of deteriorated products of the agglutinins, agglutinoids, which may have a stronger affinity for the bacteria than the agglutinins. They are called proagglutinoids. These proagglutinoids would combine with the agglutinogen of the bacteria and prevent the union of the agglutinins with this substance, thus preventing agglutination. The zone of dilution in which the agglutination fails to occur is known as the proagglutinoid zone or the prezone. This explanation has never been widely accepted, since it is difficult to see why the agglutinoids would not prevent agglutination in dilutions of the serum as well as in concentrated serum. Another explanation suggested is that the agglutinins are colloids and form a zone with the agglutinogen like the zones seen in the flocculation of one colloid by another. The prezone phenomenon in agglutination is often spoken of as the Neisser-Wechsberg reaction.[32]

### THE AGGLUTINATE

The combination between the agglutinable substance in the bacteria and the agglutinin of the serum is known as the *agglutinate*. According to Teague and Buxton and Joos the agglutinate is a new substance with properties different from those of the original components. This is not entirely accepted, and many authors regard the agglutinate as a combination of agglutinogen and agglutinin in which neither component is altered.

### THEORIES REGARDING THE MECHANISM OF AGGLUTINATION

Several theories have been suggested to explain the phenomenon of agglutination. It was first believed that the bacteria lose their motility and become sticky so that they cling to each other and form small clumps. This theory could only apply to motile bacteria and it was soon shown to be untenable, since the agglutinated bacteria revealed no loss of flagella and usually no increase of substance between the organisms. Nicolle suggested that the bacteria are *coagulated*, the outer layers sticking together under the influence of the agglutinins. In such a reaction morphological changes should occur in the organisms, but morphological changes cannot be made out in agglutinated bacteria to support this point of view.

Ehrlich taught that agglutinins are cell receptors which are cast off by the cells and appear in the blood. These receptors possess haptophoric groups which combine with haptophoric groups of the bacteria, and also

functioning agglutinophoric groups. The haptophoric groups are resistant and thermostable, the agglutinophoric group thermolabile. When the agglutinophoric groups are destroyed the haptophoric groups remain as *agglutinoid*, still being able to combine with and bind the agglutinogen of the bacteria. The agglutinins are regarded by Ehrlich as analogous to foodstuffs which possess haptophoric groups by means of which they are anchored to the cell and zymophoric groups by means of which they are prepared for digestion by the cells. Agglutinins are designated in Ehrlich's nomenclature as haptines of the second order, the antitoxins and antiferments being haptines of the first order, and the bacteriolysins haptines of the third order.

Bordet[33] believes that agglutination is a purely physical phenomenon. Since dead bacteria are agglutinated just as the living organisms, Bordet suggests that an alteration takes place in the bacteria under the influence of the agglutinin, and the bacteria, like other lifeless particles, are then precipitated. He further points out the relation of agglutination to salts and finally comes to regard a changed molecular attraction as the cause of agglutination. Bordet distinguishes two phases in agglutination: the "période d'impression" in which the agglutinins combine with the bacteria, and the agglutination proper. In the first phase there is an altered molecular attraction of the isolated particles, in the second these altered particles combine in aggregates under the influence of the salts in the serum. The first phase in agglutination resembles the combining phase in adsorption by colloids; the entire reaction may be a colloid adsorption between the colloid constituent of the bacteria and the colloid agglutinins. The influence of electrolytes in agglutination, which Bordet has demonstrated, is further evidence of the colloid nature of the reaction.

The reaction of agglutination of bacteria by agglutinins and the formation of precipitates in protein by precipitins in immune serum has been much discussed, and at present there is considerable evidence that the agglutinins and precipitins are identical, the bacteria being carried down mechanically in the precipitation. Paltauf thinks that clots may form in the immediate neighborhood of the bacteria and on their surface in the reaction between the agglutinogen and the agglutinin, just as in the clotting of fibrin the clot forms about the center around which the clot takes form. These clots might carry the bacteria down mechanically without any actual production of precipitates in the serum.

Whatever the actual mechanism of agglutination, agglutinins may be regarded as immune substances produced in the body by the introduction of the agglutinogen acting as an antigen, and able to combine with and bind this agglutinogen represented by the agglutinable substance in the bodies of the organisms.

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## Chapter X

## THE PRECIPITINS

The *precipitins* were discovered by Kraus,[1] who observed that the immune sera obtained in animals by the injection of typhoid and plague bacilli and the cholera vibrio give a precipitation with bacteria-free filtrates of these organisms, while no precipitates were produced by normal sera. These precipitins were, moreover, *specific* for the organism producing them and the reaction thus a *specific immune phenomenon.* The observations of Kraus were confirmed by Nicolle[2] and Norris[3] for other organisms, *Bacillus coli*, *Bacillus mallei*, etc. It was further shown by Ascoli[4] that precipitins could be used for the diagnosis of glanders and anthrax in horses.

In addition to bacteria other protein substances cause the production of precipitins on injection into animals. Tchistovitch[5] and Bordet[6] obtained them with horse, rabbit, and eel serum, and with cow's milk. The precipitins arising from the introduction of animal protein into animals are called *Zoöprecipitins.* Similar substances coming from plant protein are called *Phytoprecipitins.*

It was subsequently shown by Wassermann and Schütz[7] that the precipitin reaction could be used to differentiate types of animal proteins and this reaction was employed by Uhlenhuth[8] for medicolegal tests, especially in the identification of human blood. The differentiation of animal species by means of the precipitins was developed especially by Nuttall,[9] while Wells and Osbourne[10] have employed it with gratifying results in the identification of plant proteins.

Bacterial precipitins have now been obtained also against the dysentery bacillus by Dopter; against *Bacillus prodigiosus*, *Bacillus pyocyaneus* and *Proteus* by Norris; against diphtheria and pseudodiphtheria by Wassermann; against the tubercle bacillus by Kitajima, Bonome, and Ruppel; against streptococci by Marmorek; against pneumococci by Heyrovsky and Panichi; against meningococci by Dopter; against gonococci by Robinson and Meader.

### COMPONENTS OF THE REACTION

The precipitin reaction consists in a precipitation of flocculent particles in an immune serum on the addition of the antigen with which the animals have been treated. The substance in the immune serum taking part in the reaction is called the *precipitin*, the antigen employed the *precipitinogen*, while the product of the two is known as the *precipitate.*

### THE PRECIPITINOGEN

Various antigens may be used for the production of precipitins, animals or plant proteins, bacterial filtrates, etc. With bacteria the precipitinogens may be obtained from agar or broth cultures filtered through bacteria-proof filters and sterilized by 0.5 per cent. carbolic acid. Such filtrates cause the production of both agglutinins and precipitins in animals. According to Brieger the precipinogen or agglutinogen gives Millon's reaction, is easily soluble in water, insoluble in alcohol, and can be salted out with ammonium sulphate. Brieger and Mayer[11] obtained fluids containing agglutinogen, but no precipitinogen, and the identity of the two

antigens is not definitely established. Pick[12] has concluded that precipitinogens from broth cultures of bacteria are protein split products, not albumoses and not peptone. They cannot be characterized chemically. Only *colloid proteins* give rise to precipitins, and apparently other types of colloids do not cause this reaction. Split products of proteins like amino-acids and polypeptids do not give precipitins. Precipitins are not produced with crystalloids, fats, or carbohydrates.

Bacterial and animal precipitinogen has been shown by Kraus and Joachim[13] to lose its coagulability under physicochemical conditions, temperature, etc., without loss of its binding capacity for precipitin. With its loss in coagulability the altered precipitinogen has rather an *increased avidity* for precipitin.

This altered bacterial precipitinogen will still cause the production of precipitins in animals. It is analogous to toxoids and is designated *precipitoid* by Kraus.

## THE PRECIPITIN

The antibody appearing in the serum of animals treated with precipitinogen is called the *precipitin*, and bacterial precipitin may be distinguished from zoöprecipitin and phytoprecipitin. The amount of precipitin in the serum depends somewhat upon the species of animal used, somewhat upon the length of immunization, and the time of bleeding after the last injection. According to Kraus and Schiffmann[14] the place of formation in the body depends upon the region selected for immunization. With peritoneal inoculation the precipitins appear in the blood-serum and in extracts of the omentum, while with subcutaneous and intravenous dosage they appear only in the blood. Kraus believes that the precipitins in the blood-stream are probably produced in the endothelial lining of the blood-vessels. Cantacuzène[15] and Jonescu Milraisen think that the leukocyte-containing organs are the place of formation of the precipitins, especially those containing mononuclear leukocytes.

The precipitins lie in the globulins of the serum, particularly in the euglobulins, and have never been obtained free of protein. All reagents which injure the proteins of the serum injure the precipitins, such as acids, alkalies, urea, and formaldhyd. They are digested and destroyed by pepsin-hydrochloric acid and by trypsin. When heated to 55° to 60° C. bacterial precipitins lose their property of causing precipitation with bacterial precipitinogen (Kraus and von Pirquet), this property not being restored by the addition of complement. They can still combine with and bind precipitinogen. Such modified precipitins are called *precipitoids* by Kraus just as are the modified precipitinogens, but are designated *precipitinoids* by other authors (Park and Williams). Precipitins may also be converted into precipitinoids at room temperature and later lose their binding capacity for precipitinogen (Kraus and von Pirquet).

## THE PRECIPITATE

The specific precipitate arising from the mixture of precipitinogen and precipitin consists of a turbidity and flocculation of protein particles. Its amount depends upon the quantity of precipitin, the immune serum being the precipitable substance and playing a passive rôle. Pick finds that the precipitate is *protein* which comes from the immune serum, not from the precipitinogen. Welsh and Chapman[16] have shown that the weight of the precipitate varies directly with the quantity of antiserum and is independent of the quantity of antigen employed in the reaction.

When almost protein-free filtrates are employed in immunization the precipitate consists almost entirely of albumin (Pick). The quantitative relations of the precipitinogen and the precipitin in their combination to form the precipitate and the action of salts and electrolytes are closely similar to those seen in the flocculation of colloids. The reaction may be purely colloid in character with two colloid constituents.

Considerable discussion has taken place as to whether a new substance is formed in the precipitin reaction, that is, whether the precipitate has properties which are not identical with the properties of its two constituents.

It has already been pointed out that in agglutination the agglutinate is probably a new substance (Eisenberg and Volk[17]). According to Pick the precipitate has a greater resistance to the digestive ferments than either the precipitinogen or the precipitin. If this is so, a new body is probably also formed in the precipitin reaction.

The reaction generally occurs more rapidly with precipitins arising from the introduction of animal protein than from bacterial protein. With large quantities of bacterial antigen Pick has shown that precipitation may occur in half an hour, so that the reaction thus depends somewhat on the quantity of antigen. Müller[18] has observed that in the mixture of milk and lactoserum the major portion of the precipitate consists of casein. This may be carried down mechanically in the reaction however.

## SPECIFICITY

The precipitins are highly specific, reacting best with the particular protein used in immunization. They also react, but to a lesser degree, with closely related proteins. We may thus speak of *major* or *group* precipitins for the homologous protein and *minor* precipitins for closely related types. Precipitins are almost entirely the product of immunization. Occasionally *normal* precipitins may be found in the blood for certain bacteria, but these may be the result of a natural immunization. Probably a whole series is present in small quantities just as with other immune bodies. Precipitates frequently occur from the mixture of different alien sera where immunization can play no rôle. Such reactions may not be specific.

The relationship of precipitins to other immune substances in the body has been much discussed. Their reactions are so similar to the reactions with agglutinins that many authors regard the precipitins and agglutinins as identical. Since the identity of precipitinogen with agglutinogen has not thus far been definitely established it is not safe to regard the precipitins as the same as agglutinins. Ehrlich regards the precipitins as cell receptors given off to the blood and similar to the agglutinins in their structure, being "haptines of the second order." That is, they have stable haptophoric combining groups and labile precipitinophoric or precipitating groups. When the functioning precipitating groups are destroyed the haptophoric groups are left intact and are still able to combine with the protein antigens. These would correspond to the agglutinoids. As already stated the modified precipitins are designated *precipitinoids* by Park and Williams and *precipitoids* by Kraus.

Bordet regards the precipitin reaction as essentially the same as agglutination, depending primarily upon the union of antigen with antibody in a colloidal suspension, the actual precipitation being secondary.

Paltauf believes that the precipitinogenic and agglutinogenic properties of the bacterial body correspond to two different conditions of the same protein substance.

There are a number of facts which must be explained before the precipi-

tins can be regarded as absolutely analogous to the agglutinins. In agglutination, for instance, the agglutinated material or the agglutinate is made up largely of antigen, that is, the bacteria or blood-cells, the immune serum being so highly diluted that but little can be present in the combination. In precipitation the precipitate is largely derived from the immune serum. Furthermore, in the combination between the agglutinogen and the agglutinin complement is not absorbed, just as it is not absorbed in the toxin-antitoxin reaction. It has been shown by Gay[19] that the precipitate formed by the union of protein antigen and antibody (precipitinogen and precipitin) has the property of fixing complement. Later Gay[20] came to the conclusion that two antibodies are present in antisera to proteins; one related to bacteriolytic amboceptors and fixing complement; the other, the precipitin, which combines with the precipitonogen and does not fix complement. It has also been shown by Wassermann and Bruck,[21] Friedberger,[22] and Muir and Martin[23] that the property of fixing complement of protein antisera is pretty definitely independent of the combination of precipitinogen and precipitin. If this is confirmed, precipitins would be practically analogous to agglutinins in structure.

For the present precipitins may be regarded as specific immune bodies along with bacteriolysins, antitoxins, and agglutinins.

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## Chapter XI

# ANAPHYLAXIS

### GENERAL CONSIDERATIONS

The fact that animals, following the injection of a foreign serum, become sensitive to a second injection of the same serum was first definitely described by Rosenau and Anderson[1] in America and by Otto[2] in Germany. In the latter country the phenomenon is usually called the Theobald Smith phenomenon, since Theobald Smith had long recognized that guinea-pigs used for testing diphtheria antitoxin were subsequently acutely poisoned by an administration of horse serum. Soon after the publications of Rosenau and Anderson and of Otto this reaction was seen to be similar to a condition previously described by Richet[3] and named by him *anaphylaxis*. Richet observed that in the immunization of dogs with *mytilocongestin*, a toxic substance from *Mytilus edulis*, the animals exhibited an intense but momentary shock from the second dose, but subsequently recovered. Normal animals succumbed to the same dose after a long incubation period. The treated animals were thus both hypersensitive to the poison and resistant to it. According to Richet certain toxic substances were able in small doses to act as prophylactic against large doses and at the same time to *lessen* the immunity. This latter quality Richet named *anaphylactic* as opposed to *prophylactic*. While the phenomena observed by Richet were not strictly analogous to those later described by Rosenau and Anderson, the term *anaphylaxis* has come to be employed generally by workers in this field, although certain authors like Doerr and Zinsser maintain that it should be limited to the reaction produced by foreign proteins. In this latter sense anaphylaxis is a synonym for "protein allergie," the term introduced by von Pirquet to indicate the sensitiveness to foreign proteins (and depending on antibody formation).

Other early observations upon conditions in which animals become hypersensitive after the introduction of protein substances were made by Flexner, Arloing and Courmont, Héricourt and Richet,[4] von Pirquet and Schick,[5] and by Arthus.[6] Of these the most important is that of Arthus, who found that in rabbits, after the first injection of horse serum, subsequent injections led to persistent infiltration, gangrene, and ulceration at the site of injection. This is known as the Arthus phenomenon, and is now recognized to be a symptom of anaphylaxis.

The condition of heightened susceptibility to the second injection of protein substances is also known as "hypersensitiveness." The original dose of protein which brings about this condition is usually spoken of as the "sensitizing dose," while the production of this hypersensitiveness is called "sensitization." The chain of symptoms which the sensitized animals show on the reinjection of the protein is generally called the "anaphylactic shock." All the substances which bring about hypersensitiveness on injection are spoken of as anaphylactic antigens.

### SENSITIZATION

Different species of animals vary greatly in their capacity to exhibit the phenomenon of anaphylaxis or to become hypersensitive. Thus, *guinea-pigs*

become sensitive to large doses after a single injection of minute quantities of serum. Rosenau and Anderson have shown that 0.000001 c.c. of serum will sensitize guinea-pigs so that they will exhibit typical anaphylactic shock on reinjection after fifteen to twenty days. *Rabbits* are somewhat less susceptible, requiring several doses sometimes before they become hypersensitive. Friedberger[7] finds that rabbits may develop hypersensitiveness in seven to nine days after a single large dose (1 c.c.) given intravenously. Scott[8] has employed still larger doses for rabbits and found them hypersensitive in five to seven days. *Dogs* require large doses, 3 to 5 c.c. for sensitization. *Mice* are easily sensitized by small doses given intraperitoneally, .01 c.c. guinea-pig serum or .02 c.c. horse serum.

Following the first or second injection of serum there is a *latent* period or period of *incubation* before the hypersensitiveness develops. This varies with the animal and with the type of serum used. In guinea-pigs it varies from five to nine days for beef, horse, or sheep serum. With small doses, 0.0001 to 0.00001 c.c., the period of incubation may be much longer, nineteen to twenty-five days.

The condition of hypersensitiveness lasts a variable period, depending upon the animal species and upon the type of serum. In guinea-pigs sensitized to horse serum it may last for fourteen to ten hundred ninety-six days (Rosenau and Anderson), while with turtle serum it may be over after an interval of thirty days (Ninni[9]). With rabbits and dogs the hypersensitiveness lasts usually about twenty days (Scott). In man sensitized to horse serum the period of hypersusceptibility may last for six months and even longer (eighteen hundred seventeen days according to Currie,[10] five and a half years according to Allard[11]).

## THE ANAPHYLACTIC SHOCK

The condition of hypersensitiveness manifests itself by a chain of symptoms which appear on the re-injection of the protein materials. These symptoms comprise the *anaphylactic shock*. They have been investigated with great care in a number of animal species among which they differ greatly in intensity and in distribution.

In *guinea-pigs*, which are about four hundred times as sensitive to anaphylaxis as rabbits, the preliminary symptoms following the re-injection of the protein consist of restlessness, scratching at the mouth, coughing, spasmodic, irregular breathing, and a kind of general agitation with a discharge of urine and feces. These are followed by a paresis or complete paralysis with an arrest of respiration. The animals are unable to stand and first show jerky, convulsive movements. When completely paralyzed convulsions may appear, after which the animals may die or recover. The symptoms of anaphylactic shock appear in ten to forty-five minutes and death may occur in thirty to sixty minutes. Before death the temperature is depressed several degrees (7° to 13° C., Pfeiffer). The blood shows a leukopenia with a disappearance of the polymorphonuclear leukocytes and an increase in the lymphocytes, a diminution of complement or alexin, and a delay or loss of coagulability. After death the lungs show a kind of permanent distension like emphysema (Gay and Southard,[12] Auer and Lewis[11]). They do not collapse, but remain expanded, forming a cast of the pleural cavities. The walls of the bronchi are distended from the contraction of the smooth muscle. Death is due to the respiratory immobilization of the lungs, the heart beating after the action of the lungs has ceased.

With direct injection of the protein materials into the brain, explosive symptoms may appear and the animals may die in a few minutes.

In *rabbits* there is a general prostration, the animals being unable to stand and falling to one side. They may have short clonic convulsions and expulsion of urine and feces from increased peristalsis. The respirations are slow and weak, the heart action diminished with a fall in blood-pressure (Auer[14]). The coagulability of the blood is delayed. Death is due to vascular or cardiac shock, a failure of heart action of peripheral origin affecting chiefly the right side. The heart muscle is gray and stiff, very tough, and non-irritable to stimulation. Auer and Robinson regard the heart failure as the important symptom in rabbits. Non-fatal anaphylaxis, with a fall in blood-pressure, increased heart rate, and active intestinal peristalsis, is easily produced in rabbits, acute anaphylactic death only with great difficulty. The anaphylactic shock is best brought about by intravenous injection with 1 to 5 c.c. of serum four to six weeks after sensitization with two to three injections of serum at intervals of three days. If the shock is produced by subcutaneous injections there is a local reaction at the site of inoculation, edema, gangrene, and necrosis (the Arthus phenomenon).

In *dogs* the characteristic symptom of anaphylactic shock after intravenous injection is an initial and transitory rise in blood-pressure followed by a sudden fall. This symptom was originally described by Biedl and Kraus,[15] and has been extensively investigated by Eisenbrey and Pearce,[16] Robinson and Auer, and by Manwaring.[17] The pressure may drop to 80 mm. mercury and the fall is often very rapid, 25 mm. in thirty seconds (Manwaring). At the same time the animals show great restlessness, marked prostration, tenseness of the abdominal walls, and an involuntary discharge of urine. The blood-pressure gradually returns to normal in moderately sensitized dogs, but such animals may show a hemorrhagic inflammation in the large and small intestine. This condition is called "chronic anaphylaxis" by Richet and "enteritis anaphylactica" by Schittenhelm and Weichardt.[18] In acute anaphylactic shock in dogs the blood shows a leukopenia with a loss in the mononuclear leukocytes. The coagulability of the blood is delayed due to a decrease in thromboplastin, according to Pepper and Krumbharr. The heart muscle shows degenerative changes (Robinson and Auer), but these are not the primary cause of the fall in blood-pressure which seems to be due to changes in the splanchnics.

Manwaring and Crowe[19] have shown that the fall in blood-pressure does not take place in dogs with Eck fistulas in which the liver is cut off from the general circulation. This fall is thus somehow dependent on the liver function.

In dogs large doses of serum, 10 c.c., are required to sensitize and about 5 c.c. to bring out anaphylactic shock.

In other animals anaphylaxis has not been so extensively investigated. In *cats* Schultz[20] has described cardiac disturbances in hypersensitization with horse serum, but this serum is primarily very toxic to cats. Schultz and Jordan[21] have shown that white mice and rats may be sensitized to horse serum, and on reinjection show restlessness, marked irritability of the skin, involuntary passage of urine and feces with changes in temperature and blood-pressure. Anaphylactic reactions have also been observed in cows, sheep, horses, hens, pigeons, and in cold-blooded animals.

The anaphylactic shock is thus seen to be a symptom-complex involving chiefly respiration and cardiac action. As seen from investigations of Schultz, confirmed by Dale,[22] the smooth muscle of all sensitized animals shows a higher degree of contractibility than the muscle of the non-sensitized. This may explain the bronchial spasm, diarrhea, expulsion of urine, and vomiting. At the same time there is generally an increased intestinal peris-

talsis, while the secretion of the glands, especially the liver and the pancreas, is augmented. The peripheral nerves are also more highly sensitive. The local change is usually an edema which is often hemorrhagic in character.

## PASSIVE ANAPHYLAXIS

The serum of animals which have been made anaphylactic or hypersensitive by the introduction of foreign proteins when transferred to other animals renders them also hypersensitive to the same proteins. This condition is known as *passive anaphylaxis* or *passive hypersensitiveness*. It develops in a short time in the treated animals, which react after five to seven days in the same way as the actively sensitized. When an animal of the same species is thus rendered hypersensitive the condition is spoken of as *homologous passive anaphylaxis*. With an animal of a different species it is called *heterologous passive anaphylaxis*. Passive anaphylaxis lasts only a short time compared with active anaphylaxis, thirteen days according to Otto, fifteen days according to Gay and Southard, Anderson and Frost.

## ANTIANAPHYLAXIS OR DESENSITIZATION

Several substances are capable of inhibiting the anaphylactic reaction or antagonizing the materials concerned in its production. Of these a number are non-specific, preventing a reaction no matter how it is produced. Thus the injection of large amounts of sodium chlorid, atropin, or lecithin prevent the development of the anaphylactic shock which is also done away with by narcosis and by starvation. Doerr[23] and others believe that the substances or procedures which do away with the anaphylactic reaction, that is, interfere with the mechanism of response of the sensitized animal, should be called *antagonists* to anaphylaxis. If a sensitized animal develops anaphylactic shock as a result of the injection of an homologous protein it is no longer sensitive to this protein. This condition is described as *antianaphylaxis* by Besredka[24] and is often spoken of as *desensitization*. It can be produced in actively sensitized animals by the injection of serum in the period of acute hypersensitiveness, but not before this condition develops. It follows various types of introduction of the antigen, subcutaneous, intraperitoneal, intracerebral, intraspinal, and alimentary. Desensitization can also be produced in passively sensitized animals (Anderson and Frost[25]).

In desensitization, after the injection of the antigen, there is a short interval lasting from one and a half to two hours before the antianaphylactic or refractory condition develops. This condition lasts some time in actively sensitized animals, the hypersensitiveness appearing again only after weeks or months (Otto, Anderson and Schultz[26]). In passively sensitized animals it lasts a shorter period, the sensitive condition again developing in three to seven days.

Friedberger[27] thinks that the antianaphylactic condition rests upon the saturation of the substances producing the reaction (anaphylactic antibody) by the antigen which is administered for desensitization.

Specific desensitization differs in important details from the desensitization produced by the administration of drugs, etc., especially in regard to the length of time it may last.

## COMPONENTS OF THE ANAPHYLACTIC REACTION

The anaphylactic reaction is due to at least two components: the anaphylactic antigen which brings about hypersensitiveness on introduction into animals and the anaphylactic antibody produced in the body in response to

its introduction. Both these substances have been extensively investigated and there is general agreement in regard to their main characteristics. The reaction is *highly specific*, the anaphylactic shock arising from the introduction of the same protein as originally used in sensitization or one closely related to it.

**The Anaphylactic Antigen.**—Different protein substances produce hypersensitiveness on injection into animals and thus act as anaphylactic antigens. This antigen has received several names. It is called anaphylactogen by Friedberger, anaphylaxogen by Kruse,[28] sensibilinogen by Besredka.[29] In America this antigen is usually spoken of as a sensitizer or a sensitizing substance.

Sensitizing substances come chiefly from animal and vegetable proteins. They include serum (and perhaps hemoglobin), milk, egg-albumin, organ and tumor extracts, red blood-cells, leukocytes, spermatozoa, bacterial extracts, bacterial nucleoproteins, yeast cells, and possibly pollen. In general, protein materials with large molecules such as native proteins give rise to the anaphylactic reaction better than protein split products with finer molecules. Certain protein derivatives like amino-acids and polypeptids do not produce this condition. Crystallized proteins like hemoglobin and egg-albumin, and vegetable proteins like edestin, which may be regarded as chemically single substances, will act as sensitizers, however.

Little is known chemically about the proteins which will produce anaphylaxis beyond the fact that various types of proteins will give rise to the reaction. These types are the globulins of animal or plant origin, albumins, nucleo-albumins, and glycoproteins.

**The Anaphylactic Antibody.**—Following the introduction of the anaphylactic antigen the condition of hypersensitiveness develops due in general to the production of some sort of an antibody. This antibody has received various names. It is called toxogen by Richet, reaction body by Otto, albuminolysin by Nicolle, sensibilizin by Besredka, anaphylactin by Gay. It is now commonly spoken of as the anaphylactic antibody.

The anaphylactic antibody appears in the blood of the sensitized animals generally in ten to twenty days and thus follows the laws of immune body production. It resists heating to 56° C. and can be preserved for a long time without losing its activity. The amount in the blood closely parallels the amount of precipitin which is produced by animals after the injection of proteins, and also the amount of complement-fixing bodies. Little is known clearly about the origin of anaphylactic antibody, although it is believed that it is produced in the lymphoid organs like other antibodies.

## THEORIES SUGGESTED TO EXPLAIN ANAPHYLAXIS

It was originally supposed by Rosenau and Anderson and by Otto that in anaphylaxis a true antibody was produced in the sensitized animals which combined with the antigen, and in some unknown way the cells of the animals became more sensitive to the poisonous action of the combination. Subsequently several theories have been proposed to explain the phenomenon, the experiments carried out in support of these theories bringing out a number of highly important facts. Thus Gay and Southard attributed the increased susceptibility of the sensitized animal to the presence of unneutralized protein of the antigen. This is freed on the second injection and then exerts toxic action. It apparently is different from the sensitizing substance in the antigen. While subsequent investigation has not verified the original view of anaphylaxis as held by Gay and Southard, it is important to remember that these authors were the first to apply chemical

methods to the study of this protein antigen and the first to call attention to the presence of lesions in the cells of the sensitized animals.

In the view of Besredka the anaphylactic antigen contains two separate substances: the *sensitilinogen* and the *antisensibilizin*. In response to the action of the *sensibilinogen* the body of the animal produces a specific antibody, the *sensibilisin*. At the second injection the sensibilisin reacts with the antisensibilisin of the antigen. The sensibilisin being attached primarily to the cells of the central nervous system, the combination of sensibilisin and antisensibilisin would affect these cells and shock be produced by a kind of toxic action of this combination.

In bacterial anaphylaxis Pfeiffer has suggested that the treated animal produces amboceptor as a result of the first injection. On reinjection the bacteria become laden with this amboceptor and are then dissolved by the complement or alexin of the animal, poisonous endotoxins being liberated.

Gradually, the conception developed that in anaphylaxis a new substance is formed by the union of anaphylactic antigen and anaphylactic antibody, that this new substance has toxic properties, and that the anaphylactic shock, or the reaction on the reinjection of the antigen, is due to the formation of this toxic substance in the blood of the animal.

This conception of anaphylaxis rests in the main upon the thesis of Abderhalden[30] that proteolytic ferments appear in the blood after injection of proteins or protein derivatives. In anaphylaxis, it was supposed, these ferments are produced in the sensitized animal, and on reinjection of the antigen they give rise to a parenteral digestion of the protein; as a result poisonous products are liberated from the protein molecule. It had already been proved by the work of Vaughan and Wheeler[31] that a poisonous group can be extracted from proteins and efforts were therefore made to determine whether such a poison can be obtained from the substances which are known to be concerned in the production of anaphylactic shock. It had been maintained, for instance, that in anaphylaxis precipitins are usually formed. Doerr and Russ[32] now took the precipitates formed by the union of horse serum and antihorse precipitating serum, subjected them to the action of complement, and after twenty-four hours contained products which killed guinea-pigs acutely with all the symptoms of anaphylaxis.

Similar poisons were obtained by Friedmann from mixtures of beef blood laden with amboceptor and fresh rabbit serum complement. This material was centrifugalized before hemolysis occurs and produces anaphylactic poisoning in rabbits. No reaction occurs when inactive normal rabbit serum is employed instead of fresh rabbit serum. Subsequently, Friedberger[33] took the precipitate formed by sheep serum and antisheep precipitating serum from rabbit and digested it with fresh guinea-pig complement. The product gave rise to symptoms in guinea-pigs which Friedberger regarded as those of true anaphylaxis; he concluded, therefore, that anaphylactic shock is due to a poison which is liberated from the immune precipitate by the complement by a kind of enzyme action or digestion. This poison is designated *anaphylatoxin* by Friedberger. Large quantities of complement are necessary and the toxicity of the final product depends to some extent on the amount. Only complete complement suffices, the middle-piece or end-piece alone not bringing on the reaction.

Anaphylatoxins were also obtained from boiled precipitates and fresh guinea-pig serum, from erythrocytes, amboceptor and guinea-pig serum, and from sensitized bacteria, *i. e.*, bacteria laden with amboceptor and guinea-pig serum. They are not altered by heating to 55° C. for one and a half hours, but are destroyed completely at 65° C.; can be dried and preserved for a long

time, are precipitated by alcohol, may be extracted with ether and chloroform, and are not dialyzable. Both Friedmann and Friedberger teach that the anaphylatoxins, which are produced "in vitro" from the antigen-antibody combination by the action of complement, are also produced in the sensitized animal, where they give rise to the symptom-complex known as anaphylactic shock.*

It was subsequently shown that the anaphylactic poison does not arise merely from the decomposition of the antigen. Keysser and Wassermann[34] obtained such poisons from immune horse serum and insoluble substances like barium sulphate and kaolin, subjected to the action of guinea-pig complement; they came to the conclusion that immune substances in the serum, possibly the amboceptor (precipitin), furnished the matrix from which the poisons were derived. Anaphylatoxins, however, were soon obtained from fresh guinea-pig serum alone by Bordet, who permitted complement to act on ordinary agar and found anaphylactic poisons in the product. Recently Novy and De Kruif[35] have obtained toxic substances producing symptoms like anaphylaxis from guinea-pig, rabbit, and rat serum brought in contact with a variety of substances, trypanosomes, agar, etc. Blood also becomes toxic if its coagulation be delayed.

As a result of these investigations it is evident that many proteins can produce anaphylatoxins under a variety of conditions. Doerr states that any serum can become poisonous when brought in contact with *adsorbing* substances, whether these last come from precipitates, sensitized cells, bacteria, coagulated protein, Witte's peptone or agar, and the serum itself must be the source of the poison. Either a change occurs in the structure of the components of the serum or an adsorption of antagonistic substances takes place, leaving active poisons as a result.

With these observations in mind it is necessary to determine whether anaphylactoxins are actually produced in the sensitized animal at the time when the antigen is reinjected, and whether their production will explain the chain of symptoms known as anaphylactic shock. The question is still far from settled, and a number of important facts must be reconciled before final judgment can be passed. As already mentioned, it was early indicated by Schultz that the smooth muscle of sensitized animals reacts more energetically when brought in contact with homologous serum than does muscle of non-sensitized animals. This work was confirmed by Dale, who found that the uterine muscle of guinea-pigs sensitized to horse serum will react to this substance in a dilution of 1 : 2000 to 1 : 10,000, while the same muscle from non-sensitized animals fails to react at a dilution of 1 : 20 and less. After strong reactions this sensitized uterine muscle is desensitized. Normal uterine muscle cannot be sensitized by immersion in immune serum, that is, cannot be passively sensitized. Furthermore, it has been shown by Weil[36] that with guinea-pigs given large amounts of immune serum and killed in a few minutes the uterus will not react to the homologous antiserum even if the immune serum is left in the organ. The smooth muscle of the uterus in actively sensitized animals and probably other fixed cells of anaphylactic animals has an increased reactive ability for the protein used in sensitization. Furthermore, Dale has pointed out that the actively sensitized muscle reacts so quickly on the introduction of the antigen as to make it extremely improbable that antigen and antibody can unite in the cell and produce poisonous substances there by enzyme action. It can hardly be doubted, therefore,

* Deorr, Wells, and other authors believe the anaphylatoxins are preferably designated *serotoxins*, since it is now recognized that they arise from the blood-serum and not from the antigen.

that an increased reaction time in the cells of the sensitized animals for the antigens used in the sensitization is one of the cardinal symptoms of anaphylaxis or hypersensitization.

Again, no satisfactory evidence has thus far been presented that poisonous substances of the nature of anaphylatoxins are produced in sensitized animals in anaphylactic shock. As Park, Williams, and Krumwiede[37] point out, if poisonous substances in the circulating blood are the cause of the symptoms one would certainly expect that this toxicity could be demonstrated on other animals. Novy and De Kruif found that the blood of sensitized and shocked animals is moderately toxic. Weil, however, transfused normal dogs with the whole blood of dogs which were nearly dead or dying of anaphylactic shock and utterly failed to demonstrate any increased toxicity. Doerr also holds that no satisfactory proof has been brought to show that anaphylatoxins are actually produced in anaphylactic shock.

Finally, it is evident from this brief and necessarily incomplete review of the subject of anaphylaxis that the primary symptom in the manifold phenomena of hypersensitiveness is a change in the reaction capacity of the cells of the body, particularly the smooth muscle, as a result of which they react to much greater dilutions or much smaller amounts of the same antigen as was originally introduced. At the same time the blood contains some substance (called antibody) which can be transferred to other animals, and after a variable but always appreciable time is built up into the cells of this animal and renders these cells more highly susceptible to the original antigen. It may be that in the sensitization the cells are stimulated to the excessive production of the *same substances* which are present originally and which react with the antigen, causing muscular contraction for instance in smooth muscle. If this supposition be correct it is easy to understand that such substances would also be given off to the blood at certain periods and if transferred to other animals would be gradually assimilated by these cells. Experimental evidence in favor of such a view of anaphylaxis is not complete, however. It may be noted that the increased ability of cells to react to stimuli has long been recognized as of fundamental importance in immunity. This is especially marked in antitoxin production, where a slight excess of toxin brings out a greater elaboration of antitoxins in an immunized animal than in a normal animal. Further, as Kolle has pointed out in connection with acquired immunity, there is in many infections a specific biological change in resistence in the tissues and cell complexes for the infectious agent. On the same irritation which causes the production, of a small quantity of immune substance in a normal animal the immune animal produces large quantities of immune substance in a short time. As Kolle expresses it, the body cells have *learned* to produce antibodies just as the ganglion-cells of the brain have learned to react promptly to certain stimuli.

## BACTERIAL ANAPHYLAXIS

The first attempt to obtain bacterial anaphylaxis experimentally was that of Rosenau and Anderson who used cell-free extracts of *Bacillus typhosus*, *Bacillus coli*, hay bacilli, tubercle, and anthrax, and produced acute intensive symptoms in sensitized guinea-pigs. These observations were confirmed by Kraus and Doerr who proved the specificity of the reaction and its passive transference. The anaphylaxis from bacterial filtrates is by no means as constant as serum anaphylaxis. This is possibly due to the small amount of protein in the bacterial cells as compared with the amount in the serum that is usually employed. Thus Friedberger has pointed out that we can get 1 mg. protein from 0,01 c.c. serum, but that we must use a

whole agar culture to get this amount of bacterial protein. Anaphylaxis has now been obtained in guinea-pigs with the cholera vibrio, typhoid, coli, and dysentery bacilli, streptococci, anthrax, *Proteus* and *Vibrio Metchnikovi*.

There are certain fundamental differences between bacterial anaphylaxis and serum anaphylaxis which must be kept clearly in mind. In serum anaphylaxis the normal animal can stand large amounts of inactive serum without injury, while the sensitized animal reacts by shock. With bacteria normal animals are sensitive to extracts, autolysates, etc. Indeed, the bacterial protein may act on normal animals under certain conditions almost as much as on sensitized animals. Further the increase of sensitiveness as a result of treatment is slight and inconstant, that is, the bacterial protein is a poor anaphylactogen and not to be compared with serum anaphylactogen. The shock dose for sensitized animals is only slightly below that for normal animals; with the diphtheria bacillus, for example, there is no difference and we cannot speak of sensitization. Typhoid and tubercle bacilli and non-pathogenes, like *Bacillus prodigiosus* and *Proteus vulgaris*, act on sensitized animals more than on normal animals. This is shown chiefly by a proportionate increase in the instances of acute shock as compared with the instances of chronic poisoning.

Chemically the bacterial protein is generally low molecular material of the character of albumose not coagulated by heat, protamins, histon, nucleoproteins; in serum we have high molecular material like globulins and albumins. Just as certain bacteria are not good precipitinogens or agglutinogens they are not good anaphylactogens. With anthrax it is difficult to obtain anaphylaxis and practically impossible to obtain precipitins or amboceptors. With vibrios which cause good agglutinins and lysins anaphylaxis is easily produced.

Bacterial anaphylaxis is *specific*, but group reactions occur as with agglutinins and precipitins. It can also be transferred by the blood or serum of sensitized animals to other animals, this passive anaphylaxis being strictly analogous to passive serum anaphylaxis. With animals of the same species it is called *homologous* anaphylaxis, with animals of another species *heterologous* anaphylaxis. There is some evidence that bacterial hypersensitiveness can be inherited, since Kraus has found that the young of a mother sensitive to tubercle proteins have a greater sensitiveness to this protein than the young of normal mothers.

In both active and passive bacterial anaphylaxis the sensitized uterine muscle has an increased reactive ability for the antigen. This has been shown especially by Zinsser,[38] who has found that the uterine muscle of animals sensitized to extracts of the typhoid bacillus reacts to this extract, although not to the whole washed bacilli. Furthermore, the normal non-sensitized uterine muscle does not react with the typhoid extracts. The anaphylactic shock in hypersensitive animals must therefore be essentially a different phenomenon from the shock produced by bacterial extracts in the non-sensitized animals, just as serum anaphylaxis seems to be a different phenomenon from anaphylatoxin poisoning.

## THEORIES SUGGESTED TO EXPLAIN BACTERIAL ANAPHYLAXIS

It has already been pointed out that Pfeiffer regards the anaphylactic shock in immunized guinea-pigs, which occurs on reinjection of organisms, as due to the liberation of *endotoxins* from the bacteria. Vaughan[39] has explained sensitization as a liberation of toxic materials from the organisms, these toxic substances not being specific, but the same in all types of bacteria. The specific element in sensitization is the substance produced by the body and able to break up the protein in the organisms. A little later Friedberger

obtained *bacterial anaphylatoxins* from several different micro-organisms. These poisons were obtained from mixtures of bacterial amboceptor and complement, and were analogous to serum anaphylatoxins. Such bacterial anaphylatoxins have now been obtained from pathogens like typhoid and paratyphoid bacilli, non-pathogens like *Bacillus prodigiosus*, saprophytic micrococci and hay bacilli, from actinomyces and from spirochetes.

Considerable debate has taken place as to the matrix from which bacterial anaphylatoxins are formed. It was at first believed by Freidberger that they originate from the bacteria protein by a kind of ferment action or digestion brought about by the amboceptor-complement complex. Subsequently, Keysser and Wassermann substituted insoluble substances like kaolin or barium sulphate for the antigen and still obtained the anaphylatoxins from both normal and inactivated immune serum. These authors, therefore, regarded the amboceptor as the source of the anaphylatoxin. Jobling and Petersen[40] further showed that with mixtures of bacteria and serum in ordinary anaphylatoxin production the toxic substances originate from the serum by the action of the bacteria in removing the serum antiferments. The ferments remaining in the serum act on the serum protein and liberate toxic split products.

Doerr states that amboceptor is not necessary for the reaction, anaphylactic poisons arising from the combination of bacteria and fresh guinea-pig serum acting as complement. He believes that the anaphylatoxins do not come from the bacteria or immune body, but from the guinea-pig serum which is not merely complement, but contains all sorts of serum proteins. This serum may be poisonous during its formation from plasma and loses its toxicity after coagulation only when antagonistic substances are present which conceal the serum poison. Bacteria may absorb these antagonistic substances and liberate or regenerate the latent poisons in the serum. Similar results have been obtained by Novy.

It is thus evident that bacterial anaphylatoxins are practically identical with serum anaphylatoxins, and the same question thus arises in bacterial anaphylaxis as in serum anaphylaxis, namely, whether anaphylatoxins are actually produced in bacterial anaphylactic shock and whether they will explain the changed reaction which develops in the cells. The experiment of Friedberger and Nathan,[41] who believe that the conditions in the animal body are identical with those in the test-tube, bear somewhat upon this point. Friedberger and Nathan injected emulsions of bacteria into the peritoneal cavity of guinea-pigs, killed them after several hours, and found that the peritoneal exudates freed of bacteria had the characteristic anaphylatoxic properties. These experiments indicate merely, however, that anaphylatoxins can be produced in the animal body and offer no proof that they *are* produced in anaphylactic shock in sensitized animals. The opinion of Park and Williams that serum anaphylatoxins will not explain serum anaphylaxis as long as toxic substances cannot be demonstrated in the shocked hypersensitive animals can with equal force be urged in bacterial anaphylaxis. Furthermore, bacterial anaphylaxis is essentially a different phenomenon from poisoning by bacterial anaphylatoxins as is quite evident from the careful work of Zinsser. Thus, the anaphylatoxin theory fails to explain the cellular reactions in bacterial anaphylaxis just as it fails in serum anaphylaxis. The two reactions must be essentially the same, and a possible theory which fits in with all the facts is that the cells are stimulated by the bacterial protein to an excessive production of substances which, under normal conditions, react with the antigen and that these substances are at times given off to the blood and may be transferred to other animals, producing passive anaphylaxis.

## CHAPTER XII

### PHENOMENA RESEMBLING ANAPHYLAXIS

A LARGE number of intoxications in man and animals have been explained in the past on the supposition that the underlying changes are identical with those seen in true anaphylaxis and in consequence a good deal of confusion has arisen. In a recent review Wells[42] has pointed out the unfortunate results of such hasty generalizations and has laid down the criteria on the basis of which animal reactions can be judged to determine whether they partake of the character of true anaphylaxis or not. These criteria are:

1. The observed toxicity of the injected material must depend upon the sensitization of the animal, *i. e.*, the substance must not produce similar symptoms in non-sensitized animals.

2. The symptoms produced must be characteristic of anaphylactic intoxication as observed in the usual reactions with typical soluble proteins, being therefore the same with the same test animals, but differing characteristically with each species of animal.

3. It should be possible to demonstrate passive sensitization with the serum of sensitized animals.

4. It should be possible to demonstrate typical reactions in the virgin guinea-pig uterus strip.

5. It should be possible to demonstrate amelioration or prevention of the bronchial spasm in guinea-pigs by proper use of atropin and epinephrin.

6. The possibility that the observed symptoms are caused by capillary thrombosis or embolism must be excluded.

7. After recovery from anaphylactic shock there should be exhibited a condition of desensitization under proper conditions.

With these criteria in mind we may now consider the principal phenomena which have been regarded as anaphylactic in character, namely, histamin shock, serum sickness, horse protein sensitiveness, hay-fever, food hypersensitiveness, drug idiosyncrasy, and such reactions to bacterial proteins as the tuberculin, mallein, and typhoidin reactions. It is not necessary to discuss peptone shock, since so many different peptones have been used by different investigators with no knowledge of their chemical composition that the observations are practically all valueless.

### HISTAMIN SHOCK

Histamin, a product of protein cleavage from histidin, which is present in every known complete protein, produces a symptom-complex in animals almost identical with the symptom-complex in anaphylaxis. Small doses cause bronchial spasm in guinea-pigs, obstruction to the pulmonary circulation in rabbits, and a fall in blood-pressure in dogs. It also produces a local urticaria which closely resembles that in sensitized individuals. Wells

*Note.*—Most authors will agree with Wells in the criteria which must be met if any phenomena under consideration are to be regarded as examples of true anaphylaxis. It should be noted that the protein antigens, especially the bacterial, which can produce anaphylaxis, that is, can sensitize animals in small doses, frequently do produce *similar* symptoms in large doses. This similarity may be so great that it is sometimes very difficult to say whether they arise from a primary injection of antigen or a secondary injection after sensitization.

finds that the chief points in which histamin shock differs from anaphylactic shock are the failure of histamin to desensitize sensitized animals or tissues, the production of strong reactions in sensitized and desensitized uterine muscles, the failure to cause the temperature reactions and the changes in blood coagulability seen in anaphylaxis, and finally the lack of influence of quinin on the intoxication. It has been shown recently by Manwaring, Chilcote, and Hosepian[43] that in dogs histamin shock can be differentiated from anaphylactic shock; the liver, which is essential for the production of true anaphylactic shock, is not necessary for histamin shock.

## SERUM SICKNESS

Following a primary or secondary injection of foreign protein man is likely to develop a symptom-complex known as *serum sickness*. This occurs particularly in the administration of diphtheria antitoxin from horses and is associated with the proteins in the horse serum. Our fundamental knowledge of this phenomenon has been furnished by the investigations of von Pirquet and Schick. The symptoms of serum sickness consist of fever, skin eruptions, edema, slight albuminuria, enlargement and tenderness of the lymphatic glands with pain in the joints. The skin eruption varies greatly in degree from an urticaria limited to the site of the reaction to a generalized rash. Together with these symptoms a leukopenia develops, a drop in blood-pressure (Weil[44]), and a decreased coagulability of the blood. The symptoms arise after a definite incubation period which varies from one to three days. On repeated injections of serum the period of incubation is shortened, the so-called immediate or accelerated reaction of von Pirquet and Schick.

In addition to these mild cases of serum sickness, the administration of foreign serum may be followed by collapse and death in a very small proportion of cases, about 1 : 50,000 in antitoxin administration (Park, Williams, and Krumwiede[45]). The affected cases have chills and die of dyspnea.

Both types of reaction, the relatively mild serum sickness and the severe reaction with chills, dyspnea, and death, may follow the primary injection of serum. In general, the second injection of serum gives rise only to the serum sickness characterized by the transient fever, albuminuria, and urticaria.

Serum sickness was originally regarded as an antigen-antibody reaction by von Pirquet and Schick, the antigen coming in contact with the antibody in the circulation of the patient. They likened the phenomena to the production of precipitins in experimental animals. At the present time serum sickness is usually explained as an anaphylactic reaction, the patient being sensitized by a previous injection of the serum employed. While this explanation fits in with the symptomatology seen after repeated injections of serum, it is difficult to explain the effect seen after the first injection of serum on this theory unless we assume a previous sensitization of unknown character. In this connection it has been pointed out that the severe reactions with collapse and death, which occur usually after the first injection, are seen generally in people who are subject to hay-fever or horse asthma and develop attacks in the neighborhood of horses; children dying after serum administration are frequently cases of "status lymphaticus" (Park and Williams).

It has further been shown that in some instances desensitization can be carried out by the introduction of small doses of the serum, or more successfully by the administration of the serum in divided doses. Furthermore, atropin will relieve the condition.

Finally, definite antibodies (precipitins) have been found in the blood of the patients in serum sickness by C. W. Wells[46] and by Mackenzie and Longcope,[47] and the blood can produce passive anaphylaxis in guinea-pigs. There is, therefore, almost a complete analogy between this condition and anaphylaxis.

Despite the general conception of serum sickness as a manifestation of anaphylaxis certain authors, notably Coca, regard this condition as analogous to food sensitiveness and drug idiosyncrasy. It must be admitted that a large number of cases of serum sickness occur in individuals in whom there is no evidence of previous sensitization. Such cases would then be explained on the basis of a natural hypersensitiveness to the protein of a foreign serum which we know to be primarily toxic to alien species.

## HORSE ASTHMA

A few persons are susceptible to horse proteins and develop attacks of sneezing, cough, dyspnea, and coryza when brought in contact with horses. Such people give a specific skin reaction with an extract of horse dandruff. Horse asthma is now regarded as a true anaphylactic reaction due to an antigen-antibody combination, since Ramirez[48] has been able to produce passive sensitization in a normal person by transferring to him the blood of someone with the disease. Two weeks after the transfer of blood the second person developed characteristic attacks of horse asthma when in contact with horses and gave a positive skin test to extracts of horse dandruff in high dilution.

## HAY-FEVER

A considerable portion of the population of the United States is subject to the condition known as hay-fever, and the question of its etiology has both theoretical and practical importance. During certain seasons of the year, particularly in midsummer or early autumn, the affected individuals develop violent coryza with attacks of sneezing, conjunctivitis, and frequently fever and profound constitutional depression. Chronic cases may have asthma and rarely chronic bronchitis. It has long been known that the condition may be relieved by a change of climate, and there are areas in the United States to which hay-fever sufferers resort with very striking cessation of symptoms. These are especially mountainous regions, like the White Mountains of New England or places where land is almost entirely surrounded by water, as in the lower peninsula of Michigan.

The association of hay-fever with pollen was noted as early as 1873 by Blackley,[49] who produced catarrhal reactions by the introduction of pollen upon the nasal mucosa and conjunctiva; he further noted that pollen rubbed on the scarified epidermis produced a kind of urticaria. Our modern knowledge of hay-fever rests largely upon the work of Dunbar[50] of Hamburg, himself a hay-fever victim. Dunbar showed clearly that the condition is due to pollen grains, tracing it particularly to ragweed. He further developed methods for determining the number of pollen grains in the atmosphere and was able to show that these grains are carried long distances by air currents, often appearing in the air of cities far removed from regions where the pollen-producing weeds are growing. Dunbar believed that the symptom-complex in hay-fever was primarily an intoxication from poisonous substances in the pollen grains.

It has now been shown that there are quite a number of pollens in addition to ragweed which are responsible for hay-fever. These are particularly from plants which produce pollen in large quantity, and whose pollen is wind-borne like timothy hay, orchard grass, daisy, golden-rod, marsh grass, zenia, and aster.

The symptoms of hay-fever resemble in a general way the anaphylactic reactions which may be produced in animals by foreign proteins and the amount of pollen necessary to bring on the condition is so small that the resemblance of hay-fever to anaphylaxis becomes very marked. Apparently, Wolff-Eisner[51] was the first to definitely suggest the explanation of hay-fever on the basis of anaphylaxis, and since then a large amount of experimental material has been collected bearing upon the question. It has now been shown, especially by Parker,[52] that guinea-pigs may be sensitized by extracts of ragweed pollen, only, however, by fairly prolonged treatment. The uterine muscle of such animals gives a characteristic Schultz-Dale reaction to pollen extracts. According to Koessler,[53] passive sensitization can be called forth in guinea-pigs by injecting the blood of hay-fever patients. Finally, desensitization can be effected in hay-fever subjects by careful administration of extracts of the pollen to which the individual is sensitive before the hay-fever season. In many instances these treated individuals are entirely free from hay-fever, even if they remain in areas where the specific pollen is present and where they formerly suffered from the condition.

Against this explanation of hay-fever as an anaphylactic reaction following previous sensitization with a specific pollen a number of authors, notably Cooke and Vander Veer[54] and Coca[55] have urged the hereditary nature of the disease as a convincing reason for placing hay-fever among the drug idiosyncrasies. The fact that hypersensibility to pollen is inherited is everywhere admitted. It is difficult to see that this in any way *explains* the *mechanism* of the reaction, since the factor of inheritance may lie in the inherited susceptibility of the mucous membrane of certain people to pollen as a result of which hay-fever develops on subsequent introduction of the pollen grains. Further, various substances concerned in immune reactions may be *inherited*. Thus Hyde[56] has shown that the third or heat-resistant component of complement is inherited in guinea-pigs, this inheritance following Mendelian laws. The blood groups in man are also inherited according to the same laws.

The specific reaction of the skin and the conjunctiva of hay-fever subjects to the particular type of pollen which brings about this symptom-complex has great practical value as well as a theoretical importance. On intradermal injection of small quantities of pollen extract an urticarial wheal appears in five to twenty minutes surrounded by a zone of hyperemia 1 to 3 cm. in size. This reaction has been shown to follow the introduction of the same pollen which induces the attacks of hay-fever and is thus of great diagnostic importance, especially for the practice of desensitization. Where similar pollen extracts are instilled into the eye a distinct hyperemia and catarrhal discharge result; the reaction is likewise specific and of value in recognition of the pollen to which the individual is susceptible.

*Rose cold*, a condition in which the symptoms resemble hay-fever, but occur early in the year, before the regular hay-fever season begins, is now usually regarded as the same type of susceptibility, other pollen than ragweed being the exciting cause.

## DRUG IDIOSYNCRASIES

Many people are especially sensitive to the action of certain drugs and exhibit symptoms entirely lacking in others. Thus the administration of quinin will bring out an urticarial rash in some persons and this rash occurs whenever the quinin is given. Others are similarly susceptible to morphin, turpentine, oil of sandalwood, mercury, iodids, salicylic acid, and to synthetic

drugs like salvarsan. In addition to the skin reactions the susceptible individuals may have more pronounced symptoms like chills, and if the drugs are injected subcutaneously local edema and gangrene result, with swelling of the adjacent lymph-glands and pains in the joints. According to Jadasohn (cited by Sauerland[57]) the majority of instances of hypersensibility to drugs are *primary*, that is, the persons have never taken the drug before, only a few cases being *secondary* or *acquired* from repeated dosage. In general, the hypersusceptibility to drugs is a permanent reaction to a particular chemical compound and this hypersensibility cannot be conveyed to animals by the injection of serum. At the present time no adequate explanation of drug idiosyncrasy has been offered.

## FOOD IDIOSYNCRASIES

Certain individuals have a pronounced susceptibility to particular foods, developing severe and sometimes alarming symptoms when they use certain articles of diet which are harmless or beneficial to others. Thus some people after a meal containing strawberries show an urticarial rash with marked edema, particularly about the hands and face, accompanied by a good deal of constitutional discomfort. Others are hypersensitive to the meat of mussels, oysters, or crabs, showing a similar urticarial rash with wide-spread edema. Various kinds of meat, eggs, milk, and buckwheat (Smith) may also produce an intoxication in the susceptible. Children seem to be especially prone to exhibit hypersensitiveness to egg protein and cow's milk (Schloss).

Skin reactions are frequently elicited in susceptible people by extracts of the food to which they are sensitive. We know relatively little of the etiology of food idiosyncrasies. It is important to note, however, that several workers, including Rosenau and Anderson, Wells and Osborne,[58] and Schloss,[59] have shown experimentally that guinea-pigs can be sensitized to foreign proteins by way of the alimentary canal. According to Schloss sensitiveness to egg-albumin can be transferred to guinea-pigs by the serum of a patient with hypersensibility to this protein. In some cases desensitization can be effected by the careful administration of the particular food protein to which the patient is sensitive. In other instances the hypersensibility seems to be permanent, the patients developing the severe reaction whenever they take the particular food to which they are sensitive, as with strawberries. Cooke and Vander Veer have found that idiosyncrasy is inherited, the inheritance following Mendelian laws.

It is probable that we have in the group of food idiosyncrasies a variety of reactions some of which are true protein intoxications, possibly anaphylactic in nature, while others are like drug susceptibility, the persons being sensitive to certain chemical compounds in the ingested material. In this connection the classic experiments of Heidenhain,[60] who obtained lymphogogues from the normal flesh of mussels (*Mytilus edulis*), should be kept clearly in mind.

## TUBERCULIN

Following the injection of tuberculin normal animals show no apparent effects, while tuberculous animals respond by a rise of temperature, a local inflammatory reaction at the site of inoculation, and a focal inflammatory reaction at the foci of infection. Highly sensitive animals may be killed by large doses of tuberculin; a large sublethal dose may blunt the sensitiveness temporarily, while repeated small doses may bring about a tolerance or decreased reactivity. In human beings the skin reactivity may be lost in advanced tuberculous disease or in the presence of certain secondary infections of which measles is the most common example.

The general and focal response follows the subcutaneous injection of tuberculin (Koch). The hypersensitive skin reaction, first observed at the site of subcutaneous inoculation (Stichreaktion), may be elicited by intracutaneous injection (Mantoux and Moussu), scarification (von Pirquet), or inunction (Moro). A local conjunctival reaction (Wolff-Eisner, Calmette) may follow instillation of tuberculin into the conjunctival sac.

Several theories have been advanced to explain the tuberculin reaction. In Koch's[61] original interpretation the tuberculin contained substances which caused the necrosis of tissues and acted most energetically upon those which already contained the same toxic material. The systemic effects resulted from the destruction of tuberculous tissue with a subsequent liberation of poisonous substances. Von Pirquet[62] regarded the reaction as one of true protein allergy dependent upon the presence of antibodies in the system; these combine with the tuberculin as antigen, with the resulting formation of poisons which produce the reaction.

More recent investigations of the response of animals and man to the administration of tuberculin have led to the conception that the etiological factors of the hypersensitive condition of the skin differ from those underlying the general tuberculin sensitiveness. Thus Baldwin[63] has shown that treatment with pure tuberculoprotein does not produce cutaneous hypersensitiveness, but may induce an anaphylactic sensitiveness to tuberculoprotein analogous to that for other proteins (horse serum). He has found that the introduction of live tubercle bacilli separated from the host by a collodion sac also fails to induce skin hypersensitiveness. It was subsequently found by Austrian[64] that the general sensitiveness to tuberculoprotein can be transferred to other animals, that is, passive sensitization is possible with tuberculoproteins. The general reaction to tuberculin, therefore, falls in line with other anaphylactic phenomena while the skin reaction cannot be so explained.

Analogous to the tissue response to dead tubercle bacilli, shown by Prudden and Hodenpyl to approximate the tissue reaction to live bacilli, is the fact long known and recently emphasized by Zinsser,[65] that injection of killed tubercle bacilli may induce skin hypersensitiveness to tuberculin. Krause[66] has followed the development of the tissue response to live bacilli and the cutaneous hypersensitiveness to tuberculin; he concludes that the latter depends upon the formation of anatomic tubercle and may decrease or even disappear with the walling off of the focus by scar tissue. Roemer[67] has observed a period after infection when the skin reaction becomes positive while focus formation is not apparent, at least macroscopically. Lange[68] has found that focus formation can antedate skin hypersensitiveness.

Two types of skin reaction have been sharply differentiated by Zinsser, the immediate urticarial transitory reaction which occurs in guinea-pigs sensitized against proteins and the delayed type of inflammatory response (sometimes involving tissue destruction) associated with bacterial infection. Guinea-pigs sensitized with tuberculoprotein are anaphylactic to this substance, give the Schultz-Dale uterine reaction and the immediate skin response. Guinea-pigs sensitized with tubercle bacilli give the delayed skin reaction some days after infection and later the uterine reaction, while the immediate skin reaction occurs very rarely. In working with split products of tuberculoprotein Zinsser found that (1) tuberculous guinea-pigs give a delayed skin reaction with non-coagulable residue as well as with whole tuberculoprotein; (2) they cannot be satisfactorily sensitized with "residue," and (3) when sensitized with unfractionated tuberculoprotein a uterine reaction is obtained with such protein, but not with the residue.

The association of the tuberculin skin hypersensitiveness (delayed reaction) with the presence of tuberculous tissue in the animal has led Lange to investigate the possibility of inducing the skin condition by intraperitoneal injection of sterile Mandler filtrates of tuberculous tissue. Filtrates from focal reactive tissue arising from the injection of infusorial earth were used for controls. Both treatments induced moderate skin reactions to tuberculoprotein of the delayed type. Similar results have been reported by McJunkin,[69] who employed as sensitizing substance the sterile filtrates of acute peritoneal exudates induced in tuberculous guinea-pigs by the intraperitoneal injection of tubercle bacilli.

The concept that skin reactions to tuberculin are obtained only in individuals infected with the tubercle bacillus may be reconciled with the fact that large numbers of healthy adults give a positive skin test by distinguishing between the presence of anatomical tubercle which not only may cause no untoward manifestations, but actually induce a relative immunity, and the clinical condition of tuberculosis in which the foci of infection are sufficiently extensive or active to produce symptoms. The more carefully autopsies are made, the better the correlation between the percentage of individuals giving typical tuberculin skin reactions and the occurrence of anatomic tubercle.

## MALLEIN

The mallein reaction is essentially similar to the tuberculin reaction. Following the injection of extracts of the glanders bacillus infected animals respond by a rise in temperature, swelling, and tenderness about the site of injection with swelling of the adjacent lymph-glands. A local lesion may also be produced by the instillation of mallein into the eye which, in general, is a more accurate aid to diagnosis than the general mallein reaction (Schnurer[70]). The mallein reaction is usually regarded as an anaphylactic phenomenon, hypersensibility to proteins of the glanders bacillus resulting from infection with the organism.

## TYPHOIDIN

Following the introduction of the typhoid bacillus or its products, known as typhoidin, positive skin reactions develop in animals as originally shown by Gay and Force,[71] and Gay and Claypole.[72] Similar reactions may be obtained with a certain number of patients suffering from typhoid fever. According to the original ideas of Gay and Claypole the reactions went parallel with immunity. They further succeeded in producing passive sensitization to typhoidin by transferring typhoid immune serum. The observations of Gay and Claypole were confirmed by Meyer and Christiansen,[73] who concluded that the reaction was due to the union of antigen and antibody. Later, Meyer came to the conclusion that skin sensitiveness to typhoidin is rather a sign of infection than of immunity. At the present time the skin reaction to typhoidin is usually regarded as a phenomenon of anaphylaxis.

An *ophthalmic* reaction to the proteins of the typhoid bacillus has been described by Austrian.[74]

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## Chapter XIII

## THE BACTERIOPHAGE

In 1917 d'Herelle[1] reported upon a phenomenon which he regarded as new and which he named "bacteriophage." D'Herelle found that a few drops of a dysenteric stool diluted in broth and filtered had the remarkable property of *clearing* up cultures of the Shiga dysentery bacillus when added in small quantities. At the same time the organisms were killed. The dissolved culture had the further property of clearing up fresh cultures of the dysentery bacillus, and it was evident that some lytic principle was present which could be propagated along with the dysentery bacillus. In fact, d'Herelle "cultivated" this lytic principle for 935 generations. D'Herelle regarded this substance as a *living microscopic organism* which fed upon the bacteria, and he therefore named it *Bacteriophagum intestinale.* It is important to note that the lytic principle was not isolated and cultivated indepenpently, but was only kept alive upon cultures of the organism grown by regular methods. It was not transmitted by dead bacteria. Subsequently d'Herelle found similar bacteriophages for other varieties of dysentery, typhoid, and paratyphoid bacilli, *Bacillus prodigiosus*, etc., always however Gram-negative organisms.

The lytic principle known as bacteriophage is always filtrable and thus can be obtained free from the bodies of the organism. It resists the action of heat up to 65° C. and can be obtained in cultures of dysentery and similar bacilli by killing the organisms at 50° C. It is not strictly *specific*, but acts upon closely related organisms. Thus the Shiga bacteriophage acts upon the typhoid bacillus and one strain of bacteriophage from the intestinal juices acts upon a number of intestinal bacteria. Occasionally, however, a lytic principle for only one species of organism can be obtained and kept alive indefinitely on that organism. D'Herelle regards the bacteriophage as a living ultramicroscopic organism which is parasitic upon certain species of bacteria. After the publication of d'Herelle's results it was recognized that the lytic principle of d'Herelle is closely similar to one originally described by Twort.[2] Twort found that in certain agar cultures of micrococci some of the colonies could not be cultivated, but became transparent and viscid. They showed microscopically no intact organisms, but granules staining red by Giemsa. The water of condensation of such cultures when plated produced similar clear, glassy colonies. Material from these when added to pneumococci and other Gram-positive cocci also made them become transparent, and filtrates of these transparent cultures prevented the growth of fresh cultures of the same organisms. The transparent material of Twort could not be cultivated and was destroyed at 60° C. Twort believed that it was probably derived from the bacteria, although he did not rule out the possibility that it was an organism of some kind parasitic upon them. He also obtained similar dissolving material for Gram-negative intestinal bacteria.

Twort's and d'Herelle's observations upon bacteriophage have now been confirmed by a number of investigators, and it is possible to find lytic principles against a fairly large number of bacteria. The explanation of their action and their nature is by no means clear. According to Kabeshima[3]

bacteriophage is due to ferment action. This investigator believes that the bacteriophage is a catalytic agent coming from the intestinal mucous membrane which digests the bacteria by enzymes. Bordet and Ciuca[4] succeeded in producing bacteriophage against *Bacillus coli* by the intraperitoneal administration of this orgaism to guinea-pigs. The filtered peritoneal exudate, added to broth cultures of *Bacillus coli*, cleared these cultures, and material from such cleared cultures would induce the same change in fresh cultures as that produced by the original exudate. This lytic principle was also kept alive by Bordet and Ciuca for a number of generations. Like d'Herelle's bacteriophage it was destroyed at 60° to 65° C. Later Bordet and Ciuca found that when a little of the dissolved broth cultures of *Bacillus coli* was added to agar slants freshly inoculated with this organism, growth did not occur in areas where the material had been deposited, while about these areas colonies developed which soon became watery and transparent. A few colonies also grew when dissolved cultures were plated, always, however, irregular and aberrant. Bordet and Ciuca regard the bacteriophage as a variant of the organism in which an autolytic enzyme is regularly produced. Finally Zinsser[5] has shown that two types of colonies develop when cultures not completely dissolved by the bacteriophage are plated. Some of these are regular and round, and typical of *Bacillus coli;* others are irregular like those described by Bordet and Ciuca. The regular do not carry the lytic principle and give rise only to regular on further plating. The irregular carry the principle and on plating give rise to both regular colonies and irregular colonies which continue to carry the principle on further colonies. Broth emulsions of the irregular produce clearing in broth cultures in the regular way. Zinsser has found that the lytic principle is thus carried not only in broth cultures of the organism, but in the irregular colonies. Zinsser further found that bacteriophage can be obtained from glycerin extracts of the intestinal mucosa and from liver extracts. Bordet and Ciuca[6] have recently prepared an antilytic bacteriophage serum.

Weiss and Arnold[7] have recently demonstrated that bacteriophage reacts antigenically like a ferment. Arnold and Weiss[8] have shown that the antilysins (antibacteriophage) belong in the same group as antitoxins and antiferments. The phage can be precipitated like a toxin or ferment by alcohol and "toxoids" are formed by the action of heat. The antibacteriophage bodies can also be salted out of the serum containing them.

Since the fundamental observations of Twort, d'Herelle, and Bordet and Ciuca upon the phonomenon known as the bacteriophage or the Twort-d'Herelle phenomenon, a lytic substance has been found against a number of other organisms, including the cholera vibrio (Meissner[9]), the diphtheria bacillus (Blair[10]), and *Bacillus pyocyaneus* (Hadley[11]). Bacteriophage seems to be widely distributed and has been found in cultures of bacteria, in water and soil, and in the excreta of man and animals. The observations upon the phenomenon have not thus far given us any clear idea of the nature of the material which acts as a lytic agent. No therapeutic results of value have followed the use of lytic materials of this category in infectious processes.

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For a careful account of the Twort-d'Herelle phenomenon see Bull. Bacteriophage, Physiological Reviews, vol. 5 1925, p. 95.

# PART V

## THE SPIROCHETES

### GENERAL CONSIDERATIONS

THE spirochetes include the curved organisms in which a single cell occupies one or more complete spiral turns, and which have a flexuous or flexible cylindrical body. They are actively motile, the motility being due to inherent streams of protoplasm or changes in surface tension. In some instances contractile substances have been suggested. The spirochetes are separated easily from the vibrios which never have true spirals, the spiral formation being due to chains of curved organisms remaining attached after division. They are separated from the spirilla with greater difficulty, but the spirilla have rigid bodies and single or multiple true flagella. The body structure of the spirochete is different from that of the vibrios, but somewhat like the structure of the spirilla. The majority of the spirochetes are three-dimension spirals, that is, they are spiral in form from whatever side they are viewed. In certain types, like *Spirochæta Obermeieri*, they seem to be two-dimension spirals, that is spiral when viewed from one side, straight when viewed from another.

The term "spirochæta" was first applied in 1838 by Ehrenberg to a large free-living spiral organism, *Spirochæta plicatilis*, which has only recently been investigated fully. This organism remained for years as a kind of bacteriological curiosity. In 1873 Obermeier discovered a spiral parasite in relapsing fever; this was named *Spirochæta recurrens* or *recurrentis* the next year by Lebert, and *Spirochæta Obermeieri* by Cohn in 1877. About this time spiral organisms were found in the mouth by both Cohn and Koch. All these organisms were called spirochetes or spirilla indiscriminately and grouped with the bacteria. Many different types of "vibriones" had also been observed previously by Donné in his investigations of syphilis and other venereal diseases, and illustrations reproduced from Donné's original paper indicate that he saw true spirochetes.

Modern interest in spirochetes may be said to date from the entrance of the protozoölogists into this field and specifically from the work of Schaudinn and several of his pupils and followers. In 1904 Schaudinn discovered in the blood of the little owl, *Athene noctua*, a protozoan parasite, *Leukocytozoon ziemanni*, which was regarded by Schaudinn as a trypanosome and which he believed passed through a spirochetal stage in the body of an intermediate host, *Culex pipiens*. This revolutionary view would make spirochetes a part of the life cycle of trypanosomes, and as such protozoan rather than bacterial in nature. Shortly after, Schaudinn and Hoffmann discovered that syphilis, the etiology of which had baffled bacteriologists for long years, was due to a spiral organism to which they gave the name *Spirochæta pallida*. Further study of the etiological agent of syphilis convinced Schaudinn and Hoffmann that it is not a true spirochete like *Spirochæta plicatilis* of Ehrenberg, but differs from it in certain essential features. They therefore established a new genus, *Treponema*, for this class of micro-organisms. They regarded it as a protozoan parasite.

The investigations of Schaudinn and Hoffmann attracted deservedly the greatest attention, and the search for spirochetes was extended in all

directions in human and animal diseases. Within a short time many other spiral organisms were described in various pathological and normal conditions in both man and animals and occasionally in the environment. Many different varieties were found and discussion became active as to the correct interpretation of their characteristics and their proper classification.

As already mentioned, Schaudinn considered the spirochetes in *Culex pipiens* as part of the life cycle of trypanosomes, and therefore protozoan, while Schaudinn and Hoffmann regarded *Spirochæta pallida* or *Treponema pallidum* as likewise protozoan. In placing the spirochete among the protozoa Schaudinn and Hoffmann were followed by many other investigators, including especially Prowazek, Gonder, Porter, Nuttall, and Fantham, and the theory of their protozoan nature was widely accepted for the next few years. The more extended studies of the spirochetes by such observers as Norris, Novy and Knapp, Schellack, Gross, Zuelzer, Laveran, and Noguchi, and the discovery of other spiral organisms in diseases in man and animals, in mussels, and free-living in nature, revealed new facts which could not be reconciled with the interpretation of spirochetes as protozoa. In consequence the earlier views were extensively modified and spirochetes were again grouped with the bacteria. At the same time observers like Dobell were impressed with the differences between the spirochetes and the true bacteria and were inclined to group them as a somewhat distinct class of organism with affinities to the bacteria on the one hand and to the protozoa on the other hand. For this intermediate group of organisms the term "protista" was suggested and has been widely adopted.

When the spiral organisms were studied as a group and their different characteristics investigated, certain distinctive features appeared which seemed to ally them with the protozoa. The most important of these were the following:

1. A peculiar membrane or sheath in some species which resembles the undulating membrane of the trypanosomes.
2. Longitudinal division.
3. Absence of true cell wall or membrane enclosing the cytoplasm.
4. Band or ribbon-like body rather than a cylindrical body.
5. Resistance to solvent agents.
6. Resistance to plasmolysis.
7. Absence of true bacteria flagella.
8. Formation of small granules or spore-like bodies which appear in the bodies of insect carriers and which may carry the infection.
9. Presence of nuclear material.
10. Evidence of true conjugation.

1. The peculiar membrane or sheath which was originally regarded by Perrin and Schaudinn as homologous with the undulating membrane of the trypanosome and which is especially well marked on the large spiral organisms found in mussels, has now been shown by Laberan and Mesnil to be a thin, filmy structure attached to the sides of the organisms with little or no resemblance to an undulating membrane. The appearances observed by Perrin and Schaudinn were the result of fixing and staining dried preparations. In fresh and in living material this sheath is not coextensive with the organism like an undulating membrane. While it is probably an organ "sui generis," recent investigations indicate that it is not an organ of locomotion proper, but acts as a kind of rudder for the actively swimming spirochetes.

2. The spirochetes divide exclusively by transverse division. As Shellack has pointed out, the true criterion of longitudinal division is the

presence of organisms with double the thickness of the thinnest forms. This cannot be demonstrated, especially in those species where longitudinal division was claimed. The forms stimulating longitudinal division are two organisms lying side by side with part of their spirals wound about each other. Such forms frequently look like organisms dividing longitudinally. In nearly all species transverse division has now been found and the various steps observed. In general it does not occur like the transverse division of the true bacteria, but the central portion of the spiral organism thins out and the halves gradually separate, leaving a long thin terminal process attached to the ends of the newly formed cells. In certain of the larger mussel spirochetes which have a chambered or honeycombed structure, individual chambers degenerate, become quite empty of protoplasmic contents, and are broken through by the torsion of the still actively motile parts of the organism.

3. A true cell wall may be lacking in exceptional instances, as in *Spirochæta plicatilis* of Ehrenberg. In the majority of spirochetes, especially the mussel spirochetes and the small pathogenic spirochetes, a thin, but elastic membrane is present. This stains like the rest of the organism, but can be demonstrated by plasmolysis. In this connection the observations of Gross on *Saprospira grandis* are important. This organism produces peculiar bodies regarded by Gross as spores which are discharged from the body and leave behind a skeleton of the organism almost exactly like the membrane or cell wall of the bacteria.

4. The band or ribbon-like body of the spirochete, claimed for *Spirochæta balanitidis* and *Spirochæta buccalis* by Schaudinn, Hoffmann, and Prowazek, is now believed to be an artefact produced by improper methods of fixation and staining. Under proper conditions of observation the body is cylindrical and round on cross-section.

5. The resistance to solvent agent varies greatly among the spirochetes. Some organisms are dissolved quickly by such agents as saponin and sodium taurocholate, while others resist this solvent. The same difference in resistance is exhibited among the bacteria.

6. The resistance to plasmolysis also varies greatly in this group. Some organisms are exceedingly resistant to the action of fluids with different tonicity, but the contents are eventually dissolved out. In other species plasmolysis occurs in much the same way as with the bacteria.

7. Spirochetes have no flagella like those of the bacteria. At times cilia-like filaments have been stained, attached either on the ends or about the sides. The lateral flagella are now admitted to be torn portions of the outer ectoplasmic covering of the organism (periplast). The terminal processes are of two kinds (Noguchi). In some instances they represent the remnants of the periplastic covering, possibly after division. In other instances they represent prolongations of the axial filament, the central sustaining part of the organism.

8. Several different types of spirochetes break up into granules which are discharged from their bodies from time to time. Similar granules appear in the bodies of the insect vectors which produce spirochetal infections when placed upon susceptible hosts. Frequently it is difficult or impossible to find adult spirochetes in the insects, and it has been suggested by Leishman that these granules are infective and can develop into true spirochetes. This phenomenon has been investigated especially by Fantham, by Hindle, and by Balfour. All stages can be observed in fixed and stained preparations from granules to small spirochetes and from small spirochetes to adult forms. The actual development of spirochetes from granules in living

material has not thus far been seen. Until such a development can be watched, like the growth of vegetative bacteria from spores has been watched, the question cannot be regarded as settled. Furthermore, spirochetes can often be found by prolonged search in insect vectors which were supposed to harbor only infective granules (Todd).

9. Spirochetes frequently contain substances which can be stained by nuclear dyes, and from time to time a configuration of these substances like that of true nuclei has been described. Thus Perrin has noted chromatin rods and spirals in *Cristispira Balbianii*, a mussel spirochete. This so-called chromatin material is now believed to be made up of volutin or chromidial granules. It can be differentiated from true chromatin by its staining reaction and corresponds to the metachromatic granules or the Babes-Ernst bodies of the bacteria. Some of the smaller granules in the spirochetes may be true chromatin which is widely distributed among the bacteria.

10. The differentiation of spirochetes into small and active male forms and large inactive female forms by Schaudinn, Prowazek, and Porter among others, has now been found to rest upon the presence of different large and small species in the material examined. No true sexual differentiation can be made out. Some of the structures interpreted as evidence of such a differentiation have now been shown to be particles of volutin distorted in fixing and staining. The so-called blepharoplast, for example, is really a mass of volutin at the end of the organism (pole cap).

## RELATION TO BACTERIA

While the spirochetes may thus be definitely separated from the protozoa and grouped among the bacteria in the broad sense, they differ considerably in several particulars from other groups of bacteria which we have previously considered. These differences relate to internal structure, mode of locomotion, sporulation, staining reactions, and filtrability.

## INTERNAL STRUCTURE

Spirochetes vary greatly in size from minute cells less than 1/10 $\mu$ in width and 2 to 3 $\mu$ in length to large cells over 2 $\mu$ in thickness and 200 $\mu$ in length. The details of structure can be made out much more clearly in the large forms and only suggestions of the same formation can be gained from even the most exhaustive study of the small forms. For the true spirochetes (*Spirochæta plicatilis* of Ehrenberg) the unit of structure may be regarded as a delicate flexible axial filament which runs through the entire organism from one end to the other, and is spirally curved on itself. This axial filament is surrounded by a mass of cytoplasm, the periplast, which follows the curves of the axial filament and which is either composed of fibrils or which is so constructed as to give rise to an appearance of filbrillation on fixation and staining. These spirochetes have in many cases terminal projections which are not unlike the flagella of bacteria, but which, as stated above, are probably derived sometimes from the axial thread and sometimes from the periplast. Noguchi believes that the projections which show spiral arrangements like bacterial flagella are in all probability extensions of the periplast, while the more delicate projections which do not show these spirals are in all probility derived from the axial thread and connected with it by a tendenous substance. In some instances no terminal projections can be made out.

In the interior of the organism bodies are scattered here and there which take nuclear stains and are frequently regarded as chromatin. These bodies

are really volutin or chromidial threads, not true chromatin. They vary greatly in size and arrangement, but stained preparations do not necessarily indicate their exact arrangement in the living conditions.

In two genera of spirochetes, the Christispira and Saprospira, this simple arrangement of the internal structure gives place to a peculiar division of the organism into chambers, which is usually spoken of as "honey-combed." The body of the spirochete is divided by a series of transverse walls into minute spaces with protoplasmic contents. This division is not regarded as evidence of a multicellular structure, but as analogous to the chambered arrangement seen in some of the spirilla and in some of the higher cyanophyceæ, the spirillina. The walls are made up of more densely staining material, and fragments of volutin or chromidial substance are deposited at various points along them. If the organisms are plasmolyzed a single membrane is evident, not multiple cell walls.

On the outside the cristispiras possess a delicate structure known as a crista. The term "ridge" is often used to signify this structure, but it is really much more like a filmy veil. The crista is an organ "sui generis" or organella.

No definite evidence of chambered structure has been made out in the pathogenic spirochetes, but with certain of the smaller varieties structures like the crista have been occasionally observed. None of the spirochetes possess lateral flagella, the structures described as peritrichic flagella by Zettnow, Borrel, and Fraenkel now being regarded as derivatives of the external layer of the organisms, the periplast, by the maceration incident to fixing and staining.

## SPORE-FORMATION

Spores have been described in Saprospira by Gross and in Cristispira by Bosanquet. These "spores" are deeply staining bodies which develop in the chambers of these forms and are regarded by both Gross and Bosanquet as true bacterial spores. Neither the Cristispira nor Saprospira have thus far been cultivated and the interpretation of these peculiar bodies as spores rests entirely upon staining reactions. Until these organisms can be cultivated and the resistance of these so-called spores to heat and to various disinfectant or bactericidal substances which kill vegetative forms can be tested, the decision as to their true nature must be held in abeyance.

## MOTILITY

In all other groups of bacteria motility depends upon the presence of true flagella, which are endowed with a peculiar lashing movement. No such structures exist definitely among the spirochetes. The terminal projections, whether derived from the periplast or the axial thread, may assist in the movements and it is possible that the crista has a similar function. Neither terminal projections nor crista can explain the violent action of the spirochetes. This motility is difficult to describe. It consists of a rapid whirling or turning about the long axis which drives the organism forward (or backward), a kind of boring which enables the organism to penetrate obstacles like tissue cells or lumps of detritus, or to pass between them. In addition to this boring motion, the spirochetes make violent lashing movements, curling and uncurling their spirals. No satisfactory explanation of this motility has thus far been given, although several theories to account for it have been advanced. It has been suggested that the protoplasm or cytoplasm inside the organism is endowed with the capacity of streaming from one end to the other or from side to side, thus changing the surface tension.

It has also been suggested that true contractile substances are present in the cytoplasm, like the myonemes of the undulating membranes of protozoa. By their own contractions or by their change of place inside the organism these myonemes may give rise to change in form and location. Such contractile substances have not been definitely proved, although a number of observers like Noguchi have seen structures which strongly suggest myonemes.

Certain types of spirochetes, especially *Spirochæta plicatilis*, do not swim, but move by creeping along the surface of supporting objects.

Like bacteria, spirochetes have no anteroposterior polarity, that is, they move indiscriminately in either direction.

## STAINING REACTIONS

Many spirochetes stain easily by the ordinary anilin dyes, gentian-violet, carbolfuchsin, methylene-blue, etc. Others require special methods of fixation, such as the vapor of osmic acid or absolute alcohol, before staining can be effected. Nearly all of them can be demonstrated by methods useful for the protozoa, such as the Romanowsky method and its various modifications (Leishman, Giemsa). They can also be stained by hematoxylin (Delafield's iron hematoxylin, etc.).

Different spirochetes show different reactions to these dyes, the cell body and its containing structures taking up some dyes; the periplast, the terminal appendages, the crista, etc., other dyes.

## FILTRABILITY

Certain spirochetes pass through Berkefeld or Chamberland filters. This has been shown, for instance, for *Spironema recurrentis* and *Spironema Duttoni* by Novy and Knapp, and by Breinl and Kinghorn, for *Leptospira icterohemorrhagiæ* by Inada and his associates, for *Spirochæta elusa* and *Spirochæta biflexa* by Wolbach and Binger, and for *Leptospira icteroides* by Noguchi.

It has previously been pointed out that filtration involves several factors, the most important of which are the size of the pores in the material used, the pressure applied, the medium filtered, and the size and physical characteristics of the organisms. Certain bacteria pass readily through the pores of filters previously regarded as impermeable. Among the spirochetes filtration depends to some extent upon their small diameter as contrasted with their length, and possibly upon their peculiar boring movements by means of which they pass through and around objects. It is possible also that the organisms may grow through the filter if too much time is occupied in filtration. No satisfactory evidence has been brought forward to support the views of Breinl and Kinghorn, Balfour, and Fantham that spirochetes pass through filtrable stages in their development.

## CLASSIFICATION

It has now been shown that the majority of the organisms described as spirochetes differ in many particulars from the original *Spirochæta plicatilis* of Ehrenberg and various genera have been established for these other forms. In certain essentials all the forms of spirochetes resemble *Spirochæta plicatilis*, exhibiting a typical spiral arrangement. We can therefore retain the general name of *Spirochæta* for any number of the groups. The differences between the original spirochetes and the organisms found in syphilis by Schaudinn and Hoffmann were recognized immediately after the discovery of *Spirochæta*

*pallida* by the discoverers themselves, who proposed a new genus, *Treponema*, for them. Before the publication of Schaudinn and Hoffmann's new name *Treponema*, *Spironema* had been proposed by Vuillemin. The term Spironema was already in use for certain groups of protozoa and was not acceptable to those who regarded the spirochetes as protozoa. With the further evidence that the spirochetes should properly be grouped among the bacteria, the term Spironema becomes just as available as the term Treponema and has the claim of priority. The Treponemata as defined by Schaudinn and Hoffmann, however, had certain characteristics, such as ability to penetrate tissue cells, staining reactions, etc., which differentiate them from organisms like the spirochetes of relapsing fever in man and animals, all of which were included by Vuillemin in his Spironemata. Both terms may therefore be accepted provisionally, Schaudinn and Hoffmann's *Treponema* for the tissue parasites like the organisms found in syphilis and yaws, and Vuillemin's *Spironema* for the organisms in the relapsing fevers, *Spirochæta recurrens*, *Duttoni*, etc., which are essentially blood inhabitants. To what extent many other organisms described loosely as spirochetes should be placed among the Treponemata or among the Spironemata cannot be determined definitely at the present time, since only a few have been cultivated. For the present, more delicate organisms resembling *Treponema pallidum*, which are clearly tissue parasites and have certain staining reactions, reddish by Giemsa, may be put in the genus *Treponema*, the heavier organisms staining violet by Giemsa, including the blood parasites like *Spironema recurrentis*, *Duttoni*, *Carteri*, etc., as well as a number of others, which are insufficiently described and not sufficiently investigated, in the genus *Spironema*. In addition to these three genera, *Spirochæta*, *Treponema*, and *Spironema*, the genus *Cristispira* was established by Gross for the large spirochetes found in mussels, provided with a crista or ridge, and the genus *Saprospira* for similar organisms without such a ridge. Finally a new genus, *Leptospira*, was added by Noguchi for the organism of infectious jaundice described originally as *Spirochæta icterohemorrhagiæ* and the species found by Wolbach and Binger, *Spirochæta biflexa*. Since this last genus was established the newly discovered etiological agent in seven day fever, *Spirochæta hebdomadis*, has been transferred by Noguchi to the *Leptospiras*, while the organism found in yellow fever by Noguchi was described by him originally as *Leptospira icteroides*. The complete classification may be given as follows (Noguchi):

In estimating the size of spirochetes several dimensions are usually given. These are shown in the following diagram:

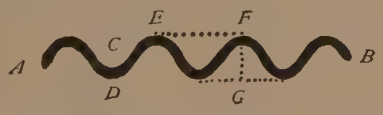


Fig. 177.—Length, *A–B;* width or diameter, *C–D;* wave length or spiral amplitude, *E–F;* spiral depth, *F–G.*

## FAMILY SPIROCHÆTACEÆ Zwellengrebel emend.

Spiral organisms in which the individual cell occupies one or more complete spiral turns. Cell body made up of an axial filament surrounded by periplast or various modifications of this structure. Terminal prolongations of periplast or of axial filament present in many species. Lateral veil-like appendage present in at least one genus. Chambered or honey-combed arrangement in two genera. No true bacterial flagella. Volutin granules

present in nearly all forms, chromidial granules in many. Probably no true chromatin. Cell bodies always elastic. Free living, saprophytic or parasitic, pathogenic and non-pathogenic.

### 1. GENUS SPIROCHÆTA Ehrenberg

Type organism *Spirochæta plicatilis* Ehrenberg.

Free living in fresh or sea-water, measuring 100 to 500 $\mu$ in length and 0.5 to 0.75 $\mu$ in thickness. Cylindrical body. Spirals regular, spiral amplitude 2 $\mu$, spiral depth 1.5 $\mu$. Large, irregular, inconstant waves. Flexible, elastic, axial filament. No chambered structure, membrane, crista, terminal spiral filament, flagella, or highly motile end-portion. Transverse division. Axial filament consists of chitin or cutin-like substance, stains violet by Giemsa or iron hematoxylin. Plasmic spirals of body stain with eosin, rubin, etc. Volutin granules present. Axial filaments resist tryptic digestion. Not dissolved by bile salts (10 per cent.) or saponin (10 per cent.). Probably always a three-dimension spiral.

Type species *Spirochæta plicatilis* Ehrenberg.
" *plicatilis* var. *marina* Zuelzer.
" *plicatilis* var. *eurystrepta* Zuelzer.
" *stenostrepta* Zuelzer.
" *gigantea* Warming.
" *flexibilis* Nägler.
" *daxensis* Cantacuzene.
" *fulgurans* Dobell.
" *minima* Dobell.

#### SPIROCHÆTA PLICATILIS Ehrenberg

Described originally by Ehrenberg.[1] Named *Spirulina plicatilis* by Cohn.[2] Investigated especially by Schellack[3] and Zuelzer,[4] from whose papers the following description is taken. Called *Spirochæta plicatilis plicatilis* by Zuelzer.

**Morphology.**—Large organism 100 to 200 $\mu$ long and 0.5 to 0.75 $\mu$ in thickness. Seldom 500 $\mu$ long. Body wound in narrow, regular spirals which are somewhat steep. According to Zuelzer it is always a three-dimension spiral. Body round on cross-section and formed of a single spiral wavy, protoplasmic layer in which a straight, axial thread runs. No morphologically differentiated membrane or cuticle about it. Plasma *alveolar*, composed of a chain of regularly placed chambers just inside an outer fibrillar periplast. These chambers are the same width as the organism, the walls being made up of a solid substance with fine granules laid on them. The inner part of these chambers is composed of a light or thin fluid which flows out if the organism be injured. The alveolar plasma shows an active streaming of the protoplasm in various directions which may cause an expansion and contraction of the body surface (Zuelzer). The stream of plasma also has a movement of rotation about the straight, elastic, axial filament. This axial filament can be seen in life passing through the entire body of the spirochete, but never sticking out of the plasma spiral. On its ends small, deeply staining granules appear. Ends round in adult forms. Freshly divided organisms have pointed ends.

**Motility.**—*Spirochæta plicatilis* has no free swimming movement, but creeps along some surface to which it may be attached. The organisms seem to grope and bore, moving restlessly forward in a sinuous manner. They often draw together quickly like muscles and creep like a screw, frequently stopping and changing direction. In moving the organisms turn about their long axis and occasionally large irregular spiral undulations develop in which the whole organism moves forward. One end may be fastened to some object, while the rest of the organism makes active, lashing movements in the free water about it. Or the middle part may be fastened and the two ends wave. In all its movements the inherent body spirals remain stable and fixed. *Spirochæta plicatilis* never stretches out to almost straight forms as "*refringens*" and "*buccalis*" do. The body is very flexible as a whole, and frequently curves or knots develop along its length.

**Staining Reactions.**—The axial thread of *Spirochæta plicatilis* stains with nuclear dyes like Delafield's hematoxylin, Heidenhain's iron hematoxylin, Meyer's hemalum, especially when they are made slightly alkaline as by adding several drops of lithium carbonate solution to Heidenhain's iron hematoxylin. The end granules stain like the axial threads. With Biondi-Ehrlich's triple stain the axial filament and the terminal granules stain light red, especially if a little dilute acetic acid be added to make the stain slightly acid. The other granules stain greenish with this dye. On staining with methylene-blue (1 : 10) and treatment with 1 per cent. $H_2SO_4$, the granules remain stained while the rest of the cell decolorizes. The small, irregularly disposed granules decolorize and may be chromatin (Zuelzer). The large granules give other volutin reactions, staining blackish with methylene-blue (1 : 10) and potassium iodid and yellow with iodin potassium iodid, and chlorzinkiodid. The alveolar plasma stains with all plasma dyes like eosin, rubin, etc. Zuelzer finds that the highly refractile granules regularly distributed in the plasma spirals stain well with nuclear dyes like hemalum and carmalum. They are better differentiated if the dyes are slightly acidified with acetic acid. Each granule lies in the upper part of a spiral. Other fine, irregular granules occur in the plasma, staining deeply with nuclear dyes. Schellack states that the walls of the alveoli stain reddish-violet with Giemsa, while their contents become light blue. With borax carmin the walls show red, the contents blue. Stains also with Loeffler's flagella stain, appearing much thicker.

**Microchemical Reactions.**—*Spirochæta plicaiilis*, both plasma and granules, is dissolved by peptic HCl in forty-eight to seventy-two hours at 40° C. and by trypsin in two to three days at 40° C. The axial filament is not attacked. Killed by saponin, not dissolved, but turned pale and shadow-like. Not dissolved by sodium taurocholate solution (1 : 10), but made pale and shadow-like. Granules but not the plasma dissolved by 0.5 per cent. soda solution. Granules dissolved in 1 per cent. $H_2SO_4$ in twelve to twenty-four hours in the cold, in 3 to 5 per cent. $H_2SO_4$ in one-half hour. The larger granules give Meyer's volution reaction (Zuelzer). The organism has no flagella and no undulating membrane. The motility seems to be due to the intracorpuscular streaming of the alveolar protoplasm which changes the surface tension. The structures described occasionally as flagella are probably the result of maceration of the fibrillar periplast. The limiting cell membrane may be a lipoid (Zuelzer) since it is dissolved by saponin. It is not visible optically.

*Division* transverse. The appearance of longitudinal division is due to the unwinding of forms tightly coiled together. A transverse band appears in the middle, cutting the organism in two. At the point of division there is a slight thickening of the axial filament which divides and breaks through with the division of the spirochete. Multiple segmentation also occurs, long organism, 130 $\mu$ or over, breaking up into a number of small forms, 10 to 11 $\mu$ long, with three to four spiral curves. (Zuelzer reports a multiplication of the granules by fission, but this has not be confirmed.)

**Distribution.**—*Spirochæta plicatilis* is found frequently in swamp water. Present in great numbers in the small lakes near Berlin where the sewage outlets drain, the edges being overgrown with vegetation and the water having the odor of $H_2S$ (Zuelzer). It has been found by Zuelzer in the mud of fresh water puddles containing decaying vegetation, in the mud of the river Spree in Berlin, in sea-water off Helgoland, and in the Gulf of Naples.

*Not cultivated on artificial media.* Zuelzer observed a multiplication of *Spirochæta plicatilis* in closed flasks, especially if $H_2S$ was added.

1. Ehrenberg: Die Infusionstierchen als volkommene Organismen. 1838.
2. Cohn: Nova Acta Acad. Caes. Leop. Carol., 1853, xxiv, P. I. 125.
3. Schellack: Arb. a. d. K. Gsndhtsamte, 1909, xxx, 379.
4. Zuelzer: Arch. f. Protistenk., 1912, xxiv, 1.

### SPIROCHÆTA PLICATILIS VAR. MARINA Zuelzer

A subspecies of *Spirochæta plicatilis* found in sea mud containing $H_2S$. Length 200 $\mu$. Thickness $\frac{1}{2}$ $\mu$. Thinner than "*plicatilis*." Has fewer, more irregular, smaller volutin bodies. Multiplication by division into two and into several individuals. Found only under anaërobic conditions.

### SPIROCHÆTA PLICATILIS VAR. EURYSTREPTA Zuelzer

A subspecies of *Spirochæta plicatilis*. Found in fresh water with it. Length up to 300 $\mu$. Thickness $\frac{1}{2}$ $\mu$. Flatter, less abrupt spirals. Not so refractile. Multiple segmentation. This form may be a developmental stage in "*plicatilis*."

### Spirochæta stenostrepta Zuelzer

Found by Zuelzer[1] always under anaërobic conditions, in fresh water mud containing $H_2S$. Thickness about $\frac{1}{4}$ $\mu$. Shows very narrow and abrupt spirals. Length up to 200 $\mu$. Average length 20 to 60 $\mu$. Long examples rare. Probably multiple division. Shortest forms pointed at both ends, 13 $\mu$ long with 11 turns. Division into two forms somewhat rare. Axial thread and volutin bodies present. Axial thread visible in living specimens. Best results in staining obtained by treating organisms before staining with trypsin or $\frac{1}{2}$ per cent. soda solution. Axial filament and granules brought out very much better. The thin forms figured in *Spirochæta plicatilis* by Schaudinn[2] as possibly male forms are really *Spirochæta stenostrepta*. Zuelzer finds no evidence that this is a male form.

1. Zuelzer: Arch. f. Protistenk., 1912, xxiv, 1.
2. Schaudinn: Deutsche med. Wchnschr., 1905, xxxi, 1665.

### Spirochæta gigantea Warming

Described by Warming in 1875. Description from Migula.

Spirals about 3 $\mu$ thick with sharply lopped off ends. Protoplasm finely granular. Each spiral is about three times as high as it is deep, the ratio being 25 to 7 to 9 $\mu$. Organism so large that by high magnification only part of the spiral can be seen. May have 16 curves. Flexible like "*plicatilis*," but not so much so.

Migula: System der Bakterien. 1900, ii, p. 1030.

### Spirochæta flexibilis Nägler

Described originally by Nägler[1] from the mud of the sewage lake in Berlin.

Differentiated from *Spirochæta plicatilis* by its smooth, even contour. Markedly flexible. Shows wavy, rolling, and corkscrew movements, turning in all directions. Ends round. Length 20 to 70 $\mu$. In preparations fixed with Flemming's solution and stained by Heidenhain or Giemsa the organisms appear to be divided into compartments, as if by proliferation. The light areas in the organism correspond to breaks in the periplast fibrils surrounding the organism and to degeneration of the axial thread. No undulating membrane. Periplast fibrils run through the surface of the ectoplasm, forming spiral threads easily seen in unstained preparations. No periplastic processes. No flagella.

The axial thread can be demonstrated by the hematoxylin method of Rosenbusch[2] as a homogeneous structure running through the center of the organism, and showing here and there a granular appearance. By Giemsa's method the periplast fibrils stain red. Longitudinal division suspected on one occasion, but not confirmed. This organism does not conform strictly to the genus *Spirochæta* of Ehrenberg. The periplast fibrils with regular spirals are so characteristic that Nägler believes the form to be a new species, possibly belonging to a new genus, for which he proposes the name *Spirophis*.

1. Nägler: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1909, l, Orig., 445.
2. Rosenbusch: Beih. z. Arch. f. Schiffs u. Tropen-Hyg., 1908, xii, Heft 5, 147.

### Spirochæta daxensis Cantacuzène

Described originally by Cantacuzène in the hot springs of Dax in water at a temperature of 52° to 56° C.

Length 30 to 100 $\mu$. Never as long as *Spirochæta plicatilis*. Thickness $\frac{1}{2}$ to $2\frac{1}{2}$ $\mu$. Actively motile. No undulating membrane. No cilia. The organism shows large and small undulations like *Spirochæta plicatilis*. The majority are fine individuals $\frac{1}{2}$ $\mu$ in diameter in which one can distinguish a sort of axial filament more chromatic than the rest of the cell. Its spiral form follows exactly the sinuosities of the organism. A certain number of coarse spirochetes occur, shorter and thicker than those of the first category, 0.5 to 1 $\mu$ in thickness. No axial filament appears definitely, but the chromatin substance forms a series of

distinct masses irregularly disposed along the body. A smaller number of cells show an enlargement in the center, measuring 0.5 to 2 $\mu$ in length, the rest of the cell measuring no more than 0.5 to 1 $\mu$ in diameter. These organisms show no chromatic spiral and no scattered masses of chromatin. The chromatic substance is concentrated in the central enlargement. Such cells stain more deeply than others. Other forms show two enlargements at either end of the central part of the organism, each containing a mass of chromatin and separated by a very thin area. This may be interrupted as a phenomenon of nuclear division and as transverse division of the cell. The organisms often form masses of filaments agglutinated in inextricable skeins.

Cantacuzène: Compt. rend. Soc. de biol., 1910, lxvii, 75.

### SPIROCHÆTA FULGURANS Dobell

Described by Dobell from the River Granta at Cambridge.

**Morphology.**—Like *Spirochæta plicatilis*, but smaller. Very long, fairly regular, and close spirals, remarkably flexible and moving with lightning-like rapidity. No antero-posterior polarity, organisms darting about in the direction of long axis with simultaneous bending, twisting, and coiling movements. Some rotation about the axis of the spiral. Length usually about 50 $\mu$, some very long, measuring 200 $\mu$. Shortest 3 to 4 $\mu$. Body cylindrical, not ribbon-like. Diameter 0.25 to 0.3 $\mu$. Diameter of spiral about 0.8 to 0.9 $\mu$. Distances equal from axis of one turn to another. Whole spiral may be thrown into a secondary spiral or succession of regular waves. Ends rounded. No flagella. No crista or undulating membrane. Organisms remain active and undergo multiplication in water containing a little $H_2S$. Kept alive three weeks in water containing Beggiotoa or sulphur bacteria. Colorless. Structureless. Stain well with iron-hematoxylin or Giemsa, black with Heidenhain, red with Giemsa. No metachromatic or other granules.

*Multiplication* by means of multiple transverse division, a long individual showing regular breaks throughout its entire length and then breaking apart into numerous short and very active forms, 3 to 6 $\mu$ long.

Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

### SPIROCHÆTA MINIMA Dobell

Described by Dobell from fresh water.

Closely resembles *Spirochæta fulgurans* in movements, method of reproduction, and habitat, differing only in its smaller size. Colorless and almost invisible under ordinary high-powered microscope. Motility extremely rapid. Length about 2 to 25 $\mu$. Diameter about 0.1 $\mu$. Diameter of spiral about 0.5 $\mu$. Smallest individuals less than 2 $\mu$ long. No flagella or crista demonstrated. Segmenting forms like those described for *Spirochæta fulgurans*.

Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

## 2. GENUS CRISTISPIRA Gross

Type organism *Cristispira Balbinaii* (Certes) Gross.

Parasitic in alimentary canals of shell-fish. Length 45 to 90 $\mu$. Diameter 1 to 1.5 $\mu$. Cylindrical waves two to five or more, large, irregular, and shallow, more numerous and regular in dying specimens. Chambered structure. Distinct, elastic, flexible membrane. Ridge or veil-like crista. Body spirally wound. No axial filament, flagella, terminal spiral filament, or highly motile end-portion. Transverse division. Membrane stains like chitin or cutin. Violet by Giemsa and light gray by iron hematoxylin. Body stains alternately red and bluish-violet by Giemsa, the crista red by Giemsa. Sharp septa and layers of chromatin granules by iron hematoxylin. Membrane resistant to tryptic digestion, but crista and chambers disappear. Crista destroyed by bile salts (10 per cent.). Body not attacked. Crista becomes fibrillar, then indistinct in saponin (10 per cent.). Body not affected.

Gross thinks that *Cristispira* is not a three-dimension, but a two-dimension spiral, wave-like in a single plane.

Type species *Cristispira Balbianii* (Certes) Gross.
" *anodontæ* (Keysselitz) Schellack.
" *pinnes* (Gonder).
" *spiculifera* (Schellack).
" *ostræ* (Schellack).
" *chamæ* (Schellack).
" *modiolæ* (Schellack).
" *limæ* (Schellack).
" *cardii-papillosi* (Schellack).
" *tapetos* (Schellack).
" *acuminata* (Schellack).
" *saxicavæ* (Schellack).
" *gastrochænæ* (Schellack).
" *pusilla* (Schellack).
" *mactræ* (Prowazek).
" *pectinis* Gross.
" *interrogationis* Gross
" *veneris* Dobell.
" *parvula* Dobell.
" *Helgolandica* Collièr.

### Cristispira Balbianii (Certes) Gross

Originally described as *Trypanosoma Balbianii* by Certes[1] from the crystalline stile of the oyster, *Ostea angulata* and *Ostrea edulis*, and subsequently from *Tapes decussatus* and *Tapes pullaster*.[2] It was later shown to be a spirochete by Laveran and Mesnil[3] and named *Spirochæta Balbianii* by Swellengrebel.[4] Transferred to the Cristispira by Gross. Found by Fantham[5] in *Tapes aureus* and by Noguchi[6] in *Ostrea virginiana*, *Venus mercenaris*, and *Modiola modiolus*.

**Morphology.**—Spiral organisms 26 to 120 $\mu$ in length and 0.5 to 3 $\mu$ in width (Mühlens[7]). The average length of young individuals just after transverse division and when still attached to each other is about 38 $\mu$, varying from 35 to 42 $\mu$, and the average thickness 1.3 $\mu$, varying from 1.1 to 1.5 $\mu$ (Schellack[8]). Laveran and Mesnil give the length as 50 to 160 $\mu$. The body of the organism consists of a series of alveoli covered externally by a flexible, spirally coiled periplast which shows contractile threads or myonemes when properly stained (Fantham). It may contain a chitinous substance (Fantham). Attached to the periplast is a delicate, fan-like membrane or ridge, the crista. It is more or less loosely attached to the body and does not extend to the ends. It is not a true undulating membrane like the undulating membrane of the trypanosomes, but is analogous to the periplastic appendage of *Spirillum giganteum* (Swellengrebel). The alveoli are produced by refractile projections from both sides of the cell-wall. These are arranged point to point as if they were imperfect cross bars. The intervals between the bars are about 3 $\mu$ (Noguchi). Numerous highly refractile granules are present, disposed along the walls and projections. The organisms are easily plasmolyzed in distilled water. The plasma escapes readily and forms round masses of the extruded protoplasm along different parts of the body. These plasmolyzed organisms show no septa. Similar results may be obtained with saponin or with 10 per cent. sodium carbonate after forty-eight to seventy-two hours. There is, therefore, no true chambered structure. Near each end the cytoplasm is concentrated into a darkly staining mass (the "calotte" of Swellengrebel). To this the crista may be attached. Ends round or blunt and slightly thinner. The organisms degenerate rapidly under artificial conditions. Occasionally terminal processes or threads have been described, but these are probably due to maceration of the fibrillar periplast. Swellengrebel believes that *Cristispira Balbianii*, *Spirillum giganteum*, and *Treponema buccale* consist of an external cellular membrane and an interior protoplasm with vacuoles. These produce an osmotic system like that described in bacteria by Fischer.

**Motility** active, the locomotion being accomplished by alternate bending, relaxing, and winding of the highly elastic, fibrillar membrane or periplast which contains some contractile substances (myonemes). The crista serves as a kind of rudder or propeller and may be attached to the contractile apparatus inside the cell. Movements corkscrew-like, slow, and

undulating, rapid, and progressive. The organisms often coil and uncoil. Porter[9] has observed pulsations actually traveling along the myoneme fibril in *Cristispira anodontæ*, a closely related species.

**Staining Reactions.**—*Cristispira Balbianii* may be stained by Giemsa or iron hematoxylin which brings out the alveolar or cross-bar structure, deeply staining blue bars alternating with faint pink areas. The crista is stained bright red, particularly along the outer free margin, and has a fibrillar structure. The cross bands which give rise to the appearance of a honey-combed structure are formed from homogeneous masses by the sudden contraction from the dehydration with absolute alcohol during fixation (Noguchi). In preparations dried in the air and fixed with methyl alcohol the banded appearance is absent. The parasite may also be stained by eosin-blue (Borrel) and tannin after fixation with absolute alcohol, by methylene-blue, by Delafield's hemotoxylin, thionin or gentian-violet after fixation with osmic acid vapor or with alcoholic Bouin's solution (picro-formol-acetic). Laveran, Mesnil, Fantham, Muhlens, and Swellengrebel regard the transverse bars as chromatin. Noguchi believes that the cross bars deeply staining with Giemsa are not chromatin, but may be volutin with minute chromidial elements.

1. Certes: Bull. Zoöl. Soc. de France, 1882, vii, 347.
2. Certes: Bull. Zoöl. Soc. de France, 1891, xvi, 95.
3. Laveran and Mesnil: Compt. rend. Soc. de biol., 1901, liii, 883.
4. Swellengrebel: Ann. de l'Inst. Pasteur, 1907, xxi, 448; 562.
5. Fantham: Parasitology, 1909, ii, 392.
6. Noguchi: J. Exper. M., 1921, xxxiv, 295.
7. Mühlens: Ztschr. f. Hyg., 1907, vii, 405.
8. Schellack: Arb. a. d. K. Gesndhtsamte, 1909, xxx, 379.
9. Porter: Arch. de zoöl. expér. et gén., 1909, s. 5, iii, 1.

### CRISTISPIRA ANODONTÆ (Keysselitz) Schellack

Described originally by Keysselitz[1] as *Spirochæta anodontæ* and obtained from the digestive tract of the fresh water mussel, *Anodonta mutabilis*, from the liver and the cast-off epithelial cells of the stomach. Found by Schellack[2] also in *Anodonta mutabilis* along with another cristispira, *Cristispira spiculifera*, by Fantham[3] and Dobell[4] in *Anodonta cygnea*.

**Morphology.**—Irregularly spiral organisms measuring from 39 to 50.9 $\mu$ in length in freshly divided individuals, average length 46 $\mu$, and from 0.9 to 1.2 $\mu$ in thickness, average thickness 1 $\mu$. Dobell gives the length as 40 to 100 $\mu$, the width constant at 0.9 to 1 $\mu$. The organisms taper very slightly toward the ends which are rounded, never pointed. The cytoplasm is chambered or alveolar inside the limiting periplast, the walls appearing as fine transverse lines, the chambers being nearly as long as broad. To the periplast is attached the crista extending almost to the end of the organism. It is a slender band-like process of the body wall (periplast), closely adherent to it and lying in the concave surfaces of the turns of the irregular spiral. It displays no visible fibrillar structure. No flagella, end appendages, spores, or basal granules can be made out (Dobell). Plasmolysis takes place in 10 per cent. NaCl. The organism ceases to move, the crista becomes detached and may break up into fibrils. By the addition of methylene-blue to the salt solution the protoplasm is stained, the crista remains colorless, and the plasmolysis is easily seen. The chromatin figures are now visible, and Dobell concludes that the nuclear figures described by earlier workers are due to preparation plasmolysis. The living organism occasionally may show one or two highly refractile granules in their protoplasm which are probably metachromatic granules (volutin).

*Motility* very rapid with a spiral or corkscrew motion of the body. The spirals of the organism remain relatively rigid during ordinary movement. The motility may be due to myoneme fibrils in the periplast (Fantham). In the living organisms Porter[6] has noted pulsations traveling along these fibrils. The cilia which have been described are probably macerated portions of the periplast.

**Multiplication** by transverse fission into two by the method of incurvation (Gross). The organism coils together, divides transversely into two at the looped end, then unwinds and straightens out, breaking into daughter individuals. Motility is retained during the whole operation. The division takes place at a transverse cytoplasmic septum. The two ends of the daughter individuals remain united for a time by the very delicate membrane or pellicle investing the body. This is subsequently drawn out very slightly, closes over the ends of the daughter individuals, which then break apart.

**Staining Reactions.**—Stains by Giemsa, the dried preparations showing the so-called "chromatin" structures, and by Heidenhain's iron hematoxylin. The crista stains pink

or lilac with Giemsa, black with Heidenhain. In organisms fixed in absolute alcohol and stained with methylene-blue the highly refractile granules are colored red.

1. Keysselitz: Arb. a. d. K. Gsndhtsamte, 1906, xxiii, 566.
2. Schellack: Arb. a. d. K. Gsndhtsamte, 1909, xxx, 379.
3. Fantham: Ann. Mag. Nat. Hist., 1907, v, 19.
———: Quart. J. Micr. Sc., 1908, lii, 1.
4. Dobell: Arch. f. Protistenk., 1912, xxvi, 117.
5. Porter: Arch. de zoöl. expér. et gén., 1909, s. 5, iii.

### CRISTISPIRA PINNÆ (Gonder)

Described originally as *Spirochæta pinnæ* by Gonder[1] from the intestinal tract and crystal stile of *Pinna aquamosa* and *Pinna nobilis*.

Closely related to *Cristispira modiolæ*. Great variations in size. The smallest measure about 10 $\mu$, the largest 50 to 60 $\mu$ in length. The thickness varies from 0.5 to 3 $\mu$ including the periplast. Average length of the recently divided individuals 30.4 $\mu$, varying from 29 to 31 $\mu$. Average thickness 1 $\mu$, varying from 0.8 to 1.1 $\mu$ (Schellack[2]). Contours parallel up to the ends which are evenly rounded. No red processes. Not so thick as other mussel spirochetes. One end is blunter than the other. Motility active, corkscrew, and progressive from either end. If one end is fixed, the other waves violently. Undulating membrane present and can be seen to glide in waves over the cell body. This membrane is easily demonstrated by various dyes, Giemsa, Heidenhain, or Hartmann's method (3.5 per cent. iron-alum) one hour followed by 1 per cent. alcoholic hematoxylin and lithium carbonate. The fibrils of the periplast are stained red by Giemsa, deep black by Heidenhain.

Gonder's view that this organism has an undulating membrane has not been confirmed.

1. Gonder: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1908, xlvii, 491.
2. Schellack: Arb. a. d. K. Gsndhtsamte, 1909, xxx, 379.

### CRISTISPIRA SPICULIFERA (Schellack)

Described originally by Schellack[1] in the crystalline stile of *Anodonta mutabilis* together with *Cristispira anodontæ* and named *Spirochæta spiculifera*. Found by Dobell[2] in the crystalline stile of *Anodonta cygnea* from the river Cam at Cambridge.

**Morphology.**—Average length of recently divided individuals 33 $\mu$, varying from 28 to 36.5 $\mu$, average thickness 0.9 $\mu$, varying from 0.7 to 1.1 $\mu$ (Schellack). Dobell finds that fixed and moist material gives average width about 0.8 $\mu$, the width varying from 0.75 $\mu$ to nearly 0.9 $\mu$. Crista always present and always terminates at either end in a minute knob-like thickening. Organisms circular in optical transverse section and have very sharply pointed ends. Both ends usually terminate in a stiff spike-like structure of variable length. This spicule appears to be a prolongation of the pellicle clothing the surface of the body. It has no independent movement and does not seem to be continuous with the crista. It is not a flagellum and is probably homologous with the end appendages visible occasionally in *Cristispira Balbianii* and other species. A chambered structure appears like that in *Cristispira anodontæ*, but more distinct. Similar appearances revealed by plasmolysis and the same chromatin figures.

**Division.**—Always transverse, never longitudinal, just as in *Cristispira anodontæ* except that immediately after incurvation the organism is much drawn out and thinned at the division point, crista and protoplasm not being divided at this stage. A little later the crista is divided and its ends knobbed. Body membrane or pellicle is still present. This minute portion of the organism, the drawn out pellicle, apparently gives rise to the terminal spicule of the daughter individuals. Freshly divided ends are sharply pointed and the body tapers markedly toward them. Spicules present a few moments after division.

1. Schellack: Arb. a. d. K. Gsndhtsamte, 1909, xxx, 379.
2. Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

### CRISTISPIRA OSTREÆ (Schellack)

Originally described as *Spirochæta ostreæ* by Schellack. Found in the crystalline stile of *Ostrea edulis* along with *Cristispira Balbianii*.

Average length of the recently divided individuals 41.5 $\mu$, varying from 38 to 42.5 $\mu$. Average thickness 1.1 $\mu$, varying from 1 to 1.3 $\mu$. Outlines converge rectilinearly just before ending in sharp points. No end processes.

Schellack: Arb. a. d. K. Gsndsamte, 1909, xxx, 379.

### CRISTISPIRA CHAMÆ (Schellack)

Described originally as *Spirochæta chamæ* by Schellack. Found in the crystalline stile of *Chama gryphoides* and *Chama sinistrorsa.*

Average length of freshly divided individuals 45.6 $\mu$, varying from 45 to 46.5 $\mu$. Average thickness 1.4 $\mu$, varying from 1.3 to 1.5 $\mu$. Outlines parallel throughout their entire length almost up to the end, then converge sharply at right angles. Points not sharp, but rounded. No terminal processes.

### CRISTISPIRA MODIOLÆ (Schellack)

Described originally as *Spirochæta modiolæ* by Schellack. Found in the crystalline stile of *Modiola barbata.*

Average length of recently divided individuals 37.5 $\mu$, varying from 36 to 40 $\mu$. Average thickness 0.8 $\mu$, varying from 0.7 to 0.9 $\mu$. Outlines parallel almost up to the ends which are evenly rounded. No end processes. Not so thick as other mussel spirochetes. Hardly twice as thick as the spirochetes of recurrent fever.

### CRISTISPIRA LIMÆ (Schellack)

Described originally as *Spirochæta limæ* by Schellack. Found in the crystalline stiles of *Lima inflata* and *Lima hians.*

Average length of recently divided individuals 37 $\mu$, varying from 35 to 41 $\mu$. Average thickness 1.4 $\mu$, varying from 1 to 1.8 $\mu$. Outlines parallel up to the ends which are evenly rounded. No terminal processes. Not easily separated from *Cristispira Balbianii.*

### CRISTISPIRA CARDII-PAPILLOSI (Schellack)

Described originally as *Spirochæta cardii-papillosi* by Schellack. Found in the crystalline stile of *Cardium papillosum.*

Easily recognized by its short compressed morphology. Average length of freshly divided individuals only about 19.1 $\mu$, varying from 18.5 to 20 $\mu$. Average thickness 1.2 $\mu$, varying from 1.1 to 1.4 $\mu$. Outlines evenly parallel up to the ends which are evenly rounded. No terminal processes.

### CRISTISPIRA TAPETOS (Schellack)

Described originally as *Spirochæta tapetos* by Schellack. Found in the crystalline stile of *Tapes decussata* and of *Tapes philippinarum.*

Separated easily from *Cristispira Balbianii*, with which it was formerly associated, by its lesser length. Average length of the recently divided individuals 34.5 $\mu$, varying from 29 to 35 $\mu$. Average thickness 1.3 $\mu$, varying from 1.1 to 1.4 $\mu$. Outlines evenly parallel up to ends which are regularly rounded. The same type of bundles of threads or fibrils on the end as in *Cristispira Balbianii* seen occasionally, probably due to maceration of the periplast.

### CRISTISPIRA ACUMINATA (Schellack)

Described originally as *Spirochæta acuminata* by Schellack. Found in the crystalline stile of *Tapes læta* with *Cristispira tapetos.*

When found with the latter species in the same numbers *Cristispira acuminata* tends to occupy the viscid fluid in the inner part of the stile. Identified by its great length and pointed ends. Average length of recently divided individuals 47 $\mu$, varying from 43.5 to 49.5 $\mu$. Average thickness 1 $\mu$, varying from 0.9 to 1.1 $\mu$. Outlines parallel almost up to the ends. Ends sharply pointed at a right angle. No terminal processes.

### CRISTISPIRA SAXICAVÆ (Schellack)

Described originally as *Spirochæta saxicavæ* by Schellack. Found in the crystalline stile of *Saxicava arctica.*

Average length of recently divided individuals 31 μ, varying from 30 to 32 μ. Average thickness 1.7 μ, varying from 1.6 to 1.8 μ. Outlines parallel up to ends which are evenly rounded. No terminal processes.

### CRISTISPIRA GASTROCHÆNÆ (Schellack)

Described originally as *Spirochæta gastrochænæ* by Schellack. Found in the crystalline stile of *Gastrochæna dubia.*

Average length of recently divided individuals 29 μ. Seldom deviates from this length. Average thickness 1.2 μ, varying from 1.1 to 1.3 μ. Contours evenly parallel up to ends which terminate on a slightly abrupt slant. No end processes.

### CRISTISPIRA PUSILLA (Schellack)

Described originally as *Spirochæta pusilla* by Schellack. Found free living in the gastric and intestinal contents of *Anodonta mutabilis.*

Similar varieties in the intestines of *Unio pictorum, Lima inflata* and *linans, Ostrea edulis,* etc. Found only rarely. Average length of recently divided individuals 13 μ, varying from 12 to 14 μ. Thickness 0.3 to 0.4 μ. Gradually tapering to fine pointed end. According to Noguchi, Bosanquet found a spirochæta 10 to 12 μ in length, which he regarded as possibly identical with *Spirochæta Hartmanni* of Gonder or *Spirochæta pusilla* of Schellack.

### CRISTISPIRA MACTRÆ (Prowazek)

Originally described by Prowazek as *Spirochæta mactræ.* A large organism like *Cristispira Balbianii,* some cells 45 μ long, 0.8 μ wide, and some 70 μ long and 1 μ wide. Found in the crystalline stile of *Mactra sulcataria* in a collection of Japanese protozoa.

Prowazek: Arch. f. Schiffs-u. Tropen-Hyg., 1910, xiv, 297.

### CRISTISPIRA PECTINIS Gross

Found by Gross in stomach and intestines of *Pecten jacobæus.* Apparently more common than other cristispiras in Gulf of Naples, 55 of 150 specimens examined, or 38 per cent., showing them.

Length 72 μ. Young individuals immediately after transverse division average 36 μ long. Thickness averages 1.5 μ. Next in size to *Cristispira limæ* and *Cristispira modiolæ* which are slightly larger. Active motility, rotation about long axis, and sinuous forward movement. Body has true spirals and not waves as Schellack has shown for *recurrens.* Number of turns in adult individuals 4, usually acute, forming an angle of 70° C. Cross-section round. Edges parallel up to ends, which are slightly rounded. Sometimes the ends are tapering or one end rounded, the other somewhat sharper. On one side of the body a very delicate ridge is seen, stretching over all the spirals, but stopping before the end. Height 1 μ. immeasurably thin. On cross-section it seems to form a direct elongation of the radius of the organism. It appears during life as a fine line, barely visible. This is the structure previously described as an undulating membrane, an organ "sui generis." Now named *crista.* It lies on one side of the organism and does not surround it spirally as with the other cristispiras. It follows the spirals, but remains always on the inner side. In life it appears as a thin band or thread, making spiral turns similar to those of the body, but separated from it. No end-processes or end-bundles. No structure to be made out in the crista.

Preparations fixed with Flemming's solution show body and crista structureless and homogeneous, with pole caps on the ends of the body. With Giemsa the body stains evenly pale blue, the pole caps appearing as clear red points. Crista stains red. Body stains blue-black with iron hematoxylin, the pole caps black or dark blue-gray.

In iron-alum solution the crista takes the color more than the body and appears deep black. Later it gives up the hematoxylin and takes up the eosin. The crista never quite reaches the pole caps. In stained preparations it appears as a simple line or a slim band.

No definitely stained periplast but a fairly thick membrane staining black with iron hematoxylin. Sublimate preparations show the body somewhat smaller than when fixed with Flemming solution. Crista somewhat shrunken. Body now seen to be divided into a number of chambers separated by cross walls like those described by Gonder, Swellengrebel, and Schellack for other cristispiras.

These chambers are not an evidence of multiple cells, but a kind of honeycomb. The body consists of a single series of chambers surrounded by a thick membrane and separated from each other by cross walls. These appear in sublimate preparations as simple lines separated from the rest of the plasma by dark staining. Occasionally individual chambers appear dark. Size of chamber varies, the number corresponds to the length of the cristispira and varies from 30 to 79. The adult cristispira may be 72 $\mu$ long, recently divided individuals 36 $\mu$ long. Height of cylindrical chamber about 1 to 1.5 $\mu$, about the thickness of the living cristispira. In dried specimens the crista is gone or left as a striped or macerated structure. Stained by Giemsa the chambered structure is clearly made out. Walls appear thickened and take the red components of the dye. Occasionally the entire chambers stain red, but remain lighter than the walls. Sometimes these red areas include several chambers. Pole caps appear thicker with iron hematoxylin and eosin. If the hematoxylin is taken out only partially the whole cristispira appears like a black staff broken up occasionally by pale red areas. With more advanced decolorization the chambers appear clearly, the walls thick, dark blue-gray to black. Occasionally the entire chamber retains the color of the hematoxylin. Pole caps thickened. With longer action of the iron-alum solution, the walls of the chambers and the pole caps become normal in thickness, and the chambers then appear bright rose.

*Transverse division* by a method hitherto not described in spirochetes. It begins by one end bending like a hook and hanging close to the body. The bending increases, the end gliding along the body till it reaches the other end. This takes one-half to one hour. During this period the cristispira makes sinuous movements, but does not change its location. The whole body is now bent double and a slight indentation appears at the bend of the organism. This gets deeper and deeper and cuts the organism into two halves. After division the organisms remain parallel and make similar movements so that one gets an impression as if longitudinal division were occurring. The crista also divides, the division beginning when the two ends of the body are parallel. The segmentation of the organism may take place through a chamber itself or between two chambers. Pole caps are not individual structures, but merely modified walls of the terminal chamber. After division the cristispira may have pole caps on one or both ends. If the segmentation goes through a chamber wall, then the two daughter-cells would have pole caps on both the new ends. If segmentation goes between chamber and wall, then one daughter-cell would lack a pole cap at point of segmentation. If chamber divides in two then daughter-cells would have no pole caps at the point of segmentation. Usual method is for chamber wall to be cut in two. When pole caps are lost in division they may regenerate.

To the process of folding together the term "incurvation" has been applied by Gross. *Cristispira pecinis* divides by transverse division after incurvation.

Gross: Mitt. a. d. Zoöl. Stat. zu Neapel, 1910, xx, 41.

### CRISTISPIRA INTERROGATIONIS Gross

Described by Gross from the stomach and intestines of *Pecten jacobæus*. Much smaller than *Cristispira pectinis* and found but rarely.

**Motility** very active. Length difficult to measure, since the ends are rolled up. Longest adult individuals about 25 $\mu$ long. Width $\frac{1}{2}$ $\mu$. Spirals number about three, but longest individuals do not always have the most spirals. Ends drawn out in fine points. After fixation with Flemming's solution the crista appears, but it is difficult to see on living specimens. On staining with iron-alum and eosin the crista is red, the body blue-black up to the pointed ends which take on the color of eosin like the crista. One gets an impression as if the crista and the ends had the same affinity for eosin. With dried specimens stained by Giemsa the body stains violet-red, the points bluish. Often the body does not take the same tone of color, but shows irregularly disposed shorter or longer bluish areas. With double staining by iron hematoxylin and eosin, if the preparations are left in iron-alum only a short time after being stained with hematoxylin, the body appears black-blue up to the red pointed ends. The picture is the same as in the preparations fixed with Flemming except for the lack of a crista. If the iron-alum acts longer, then the darkly stained body is broken in several regions by light rose-colored areas, the number of which increases with longer differentiation until the whole body apart from the red-colored pointed ends, seems made up of alternating blue and rose colored parts. The blue tone can stretch over relatively long areas or can be shown by various parts of about the same length. With still longer dif-

ferentiation the whole body stains rose-colored, the ends remain reddish, and instead of showing relatively wide bluish cross bars it reveals only delicate dark lines. The body is evidently made up of a number of chambers, 36 to 38 in the longest specimen examined. Since the longest individuals are only 25 $\mu$ long each chamber not quite a micron in length. No pole caps in the long pointed ends. Most characteristic is the peculiar and frequent rolling up of the ends. In small individuals with only about one spiral the ends are actually rolled or turned in, in opposite directions. This makes a figure like an interrogation point. With longer specimens both ends may be rolled in the same direction, that is, one end must have unrolled and then rolled up in the other direction.

Gross: Mitt. a. d. Zool. Stat. zu Neapel, 1910, xx, 41.

### CRISTISPIRA VENERIS Dobell

Described originally by Dobell[1] as a large species of cristispira from the crystalline stile of *Venus casta* (*Meretrix casta*).

Stains by Giemsa or Leishman's modification of Romanowsky after fixation of moist films by osmic vapor and absolute alcohol. Also by Giemsa after fixation in absolute alcohol. Varies in length from 50 to 60 $\mu$, average breadth in fixed and stained specimens about 1.5 $\mu$. Breadth varies from 1 to 1.9 $\mu$. Sometimes dried specimens fixed with Giemsa are almost 2 $\mu$ wide. Longest undivided individual noted was 74 $\mu$ in length. Cylindrical body, not band-like or ribbon-like, round on cross-section. In making films it may be somewhat flattened. Actual uniform diameter of the living organism approximately 1.2 $\mu$.

Body spiral shaped like a corkscrew. Approximately four complete turns in a full grown individual, two to three in recently divided specimens, and five to six in dividing individuals. Ends bluntly pointed and body tapers slightly toward the two ends. Polar caps which are visible in *Cristispira Balbianii* and *Cristispira pectinis* were not observable in *Cristispira veneris*. These appear to be confined to species which has rounded ends. No structures like flagella seen. Body flexible. Movements like those of *Christispira Balbianii* as described by Perrin.[2]

The crista is in the form of a narrow band radially situated on surface of the organism and spirally disposed. It does not often reach the extreme ends of the organism and appears to be a simple prolongation of the membrane which clothes the body. At ends it gradually merges into the body. No structures in it comparable with basal granules or blepharoplasts. Protoplasm homogeneous. No fibrillar structure in living or properly fixed specimens. Stains pink or violet by Giemsa, the body of the organism blue. No thickened, chromatic edge. In macerated specimens it is enlarged and distorted and has a very definite fibrillar structure. This is an artefact since normally it appears homogeneous. It may indicate that the crista is really composed of fibrils arranged longitudinally. Fibrillar appearance not the normal appearance of the crista. In brief the crista of *Cristispira veneris* is a delicate uniform band-like appendage wound spirally around the body extending almost to the ends. It is always present. It has no resemblance to the undulating membrane of trypanosomes. It apparently serves as a rigid lateral fin-like extension of the body in the performance of the screw-like movements of the organism.

The protoplasm appears homogeneous in the living organism, but in stained specimens has a distinct and highly characteristic arrangement. In properly prepared material the whole organism shows a protoplasmic structure consisting of a single row of chambers or alveoli. The walls are stained deep blue, their contents uniform pale blue. The chambers vary in size and appear square or oblong. The alveolar walls appear as transverse septa in optical section. Where the transverse septum joins the wall of the organism dark purple granules can be seen. These granules vary in size and always lie at edges of the organism. If the organisms are first dried and fixed in absolute alcohol, then stained by Giemsa's method, they show the remarkable "chromatin" configurations described by Perrin and others. The chambers are indistinct and vacuoles are present. Red chromatin structures may be seen in different individuals and at different points in same individual. They are often called "nuclear." Similar red granules, varying in size and distribution, appear when the organism undergoes plasmoptysis. Where the cell membrane has burst, protoplasm flows out and is seen to consist of two different substances, bluish or lilac-colored and denser dark red.

The whole cell may be compared with a jointed bamboo stem or hollow cylinder divided into a single series of chambers by transverse disk-like partitions. The cytoplasm forming the walls of the cylinder and the disk-like partitions is dense and less deeply staining. The only other morphological constituents of the cells are granules arranged around the circumference of the disk-like partitions in the dense cytoplasm which lines the cell. These granules stain red and in plasmoptysis give rise to the peculiar rods, droplets, and transverse bars of chromatin. The granules stain deep blue after long exposure to osmic vapor, purple

after brief exposure, red when osmic fixation is omitted and only absolute alcohol employed after previous drying. Prolonged action of osmic vapor so changes the granules that they are unable to take up the red staining element in the Romanowsky stain. The granules are different from cytoplasm substance which stains red with Giemsa. Probably represent a chromidial nucleus. Granules composed of chromatin and nuclear substance. More deeply staining areas along the organism are probably a thickening of the septa and a hollowing out process for the formation of new chambers in freshly divided individuals. New formed chambers are more darkly stained.

**Division** always transverse, never longitudinal, and just like that described as incurvation by Gross[3] for *Cristispira pectinis*. Long individuals about to divide consist of spirals of five to six turns. Before dividing they bend themselves double, the two halves becoming intertwined. Now transverse division begins, occurring in the middle of the curved individual at a point where a transverse partition separates two adjacent protoplasmic chambers. The partially divided organism untwists itself, returning to the form of original spiral. Fission now completed and two daughter individuals appear, short end consisting of only two to three spirals. The crista also divides. Division except for incurvation like that for bacteria and spirilla.

The body of the cristispira is bounded by a cuticle-like covering or cell membrane, the periplast of Perrin, not like the cell membrane of a trypanosome, although the same name is used for both. It may be demonstrated by plasmolysis. Also shown by Swellengrebel[4] for *Cristispira Balbianii*. Periplast not fibrillated, the fibrils being really derived from the crista. Cell membrane has no structure. (Dobell has the same interpretation of it as Gross.)

This form described by Dobell is probably identical with the cristispira obtained by Zuelzer[5] from *Venus verrucosa*.

1. Dobell: Quart. J. Micr. Sc., 1911, lvi, 507.
2. Perrin: Arch. f. Protistenk., 1906, vii, 131.
3. Gross: Mitt. Zool. Stat. zu Neapel, 1910, xx, 41.
4. Swellengrebel: Ann. de l'Inst. Pasteur, 1907, xxi, 448.
———: Centralbl. f. Bakteriol. [etc.], 1909, xlix, 529.
5. Zuelzer: Arch. f. Protistenk., 1912, xxiv, 1.

### CRISTISPIRA PARVULA Dobell

Found by Dobell in the crystalline stile of a Ceylon lamellibranch, *Venus (Meretrix) casta*. Similar and probably the same organism found in *Soletellina acuminata*.

Smallest species of cristispira investigated by Dobell. Length 20 to 45 $\mu$. Breadth in osmic-fixed specimen stained by Giemsa varies from about 0.4 $\mu$ to slightly over 0.5 $\mu$. Motile. Crista present in properly fixed individuals. In dried Giemsa preparations it was often seen to be split up into fibrils. Giemsa also reveals the so-called "chromatin" structures produced by preparation plasmolysis. Normal chambered structure of cytoplasm clearly seen in individuals carefully fixed by osmic vapor and absolute alcohol. Ends very sharply pointed. No spicules observed. No stages of division noted.

Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

### CRISTISPIRA HELGOLANDICA Collier

Described by Collier from the abdominal cavity of *Asterias rubens* in the North Sea.

**Morphology.**—Spirals from 12 to 68 $\mu$ long, average diameter 1.5 $\mu$, somewhat longer in fixed preparations. The longest individuals have about six spiral turns, each about 11 to 12 $\mu$ long. The spirals have an angle of about 75 degrees, and are thus fairly steep. Cross-section round. Points slightly rounded. Spirals parallel. Crista is about 0.75 to 1 $\mu$ high, lies on one side and seems to be structureless. No end-processes. Occasionally very fine granules of "chromatin" are visible along the inner walls. Not homogeneous as Bosanquet and Cecil believe and no chambered structure such as Fantham describes. Collier states that this species seems to have a multiple-celled body, surrounded by a stiff membrane. The crista is attached to this like a fine membrane of plasma.

**Multiplication.**—No longitudinal division as described by Perrin, Keysselitz, Gonder, Fantham, and Porter. Incurvation observed at time of transverse division. Another type of multiplication also made out consisting of delicate constrictions at the limits of the individual chambers, which deepen until the cristispira is broken up into a series of long portions. Not clear whether the crista persists along all these portions, but Collier believes

that it probably does. These portions may represent the individual elements of which the parasite is composed. These portions can now develop into new parasites as shown by inoculation of parasite-free asterias with the long segments. No spores.

Collier: Centralbl. f. Bakteriol. [etc.], 1921, cxxxvi, 132.

### 3. GENUS SAPROSPIRA Gross

Type species *Saprospira grandis* Gross.

Free living in foraminiferous sand. Measures 100 to 200 $\mu$ in length and 0.8 $\mu$ in thickness. Large, inconstant, shallow, irregular waves 3 to 5 in number. Sometimes almost straight. Chambered structure. Distinct, flexible, elastic membrane. No axial filament, crista, terminal spiral filament, flagella, or highly motile end portion. Division transverse.

Other species *Saprospira nana* Gross.
" *flexuosa* Dobell.

#### SAPROSPIRA GRANDIS Gross

A free living, spiral organism found by Gross in foraminiferous sand near the Gulf of Naples. Kept alive in cultures of drops of sea-water on slides seven to eight days, but subcultures not successful.

Organisms in cultures form star-shaped conglomerates. Colorless, thread-formed, spirally wound cells, varying in length from 2 to 100 $\mu$. Length of individual spiral, from summit of one curve to summit of next curve, 6 to 6.5 $\mu$. The longest individuals show 15 spirals, the shortest only one. Thickness about 0.8 $\mu$. Spirals narrow and acute in living material, flatter in fixed preparations. Fixed with sublimate or picric acid, the spirals are stretched to almost a straight line. Body round.

Movements not very active. They consist of slow turning about long axis, lateral bending, and slow gliding backward and forward like the movement of the oscillaria. All movements are very slow, and yet may be compared with the rapid and powerful movements of the cristispiras and spironemata.

Body divided into a series of end-to-end chambers by numerous transverse walls, which can be clearly seen. The number of these cross partitions varies, but there are usually three to four in each spiral. Often in the large chambers obscure light-colored broad-striped bars lie between the dark, almost black, sharply-marked walls. Sometimes these extend only partially through the chambers. Sometimes a chamber has a round granule in the middle instead of a cross stripe and this may look not unlike a nucleus. It represents really a process of division in the individual chamber. A new partition appears first in the middle of a chamber as an obscure broad transverse stripe. This gradually becomes clearer and sharper, showing a dark line which becomes rapidly thicker and divides the chamber into two daughter chambers. This division of the chambers may occur in any portion of the body. Structures like the pole caps of the cristispira may appear at one or both ends or may be entirely lacking. Division indicated by the rapid increase in numbers of the organism in cultures, but mode of division not definitely observed. It must be very rapid.

Since the diameter of the organisms remains constant and the length varies greatly the multiplication is in all probability by transverse division. In young cultures many individuals show only seven to eight spirals, about half the maximum number. The frequent appearance of much shorter forms with only one spiral indicates that those with seven to eight spirals may divide again before they grow out into long forms. Probably the saprospira divides at the junction of the chambers into two or more daughter-cells with an increase of the cross partition in size as a preparation for division. In addition to transverse division, in older cultures after the increase in number has reached its height, another mode of division occurs. This starts as a development of one or more bright, almost colorless chambers, the same width as the rest of the organism or slightly wider. Often these chambers appear empty, free from all plasmatic contents. They indicate the site of the new transverse division which may take place in two ways. When the empty chambers are thicker than the rest of the cell division occurs by a break through one of the walls of the empty chamber, which then appears at the end of the new organisms. More frequently the chambers have the same width as the rest of the organism, and here division begins as a slight indentation of the walls which show peculiar longitudinal stripes. Gradually the indentation deepens until the division is complete, each daughter-cell having half an empty chamber at the new ends. This mode of division is probably to be explained by the active rotation of the saprospira about the empty chambers, which naturally cannot take part in the movement and are eventually broken through in the middle.

In cultures seven to eight days old transverse division becomes less frequent and a third form of multiplication occurs, the formation of spores. These appear as one or more darkly stained areas, with the cross partition between the chambers enclosing them somewhat thickened. The dark staining of the chambers may be due to the thickening of the walls. At the same time slight indentations appear at the junction of neighboring chambers and gradually cut through the organism, dividing it up into spores corresponding in number to the number of chambers. The transverse section of the new saprospira is much less than before these spores have formed, and the form containing spores looks almost like a different organism. In the spore formation a condensation of the contents and a diminution in volume may develop with the thickening of the wall. In some instances the spores escape from the chambers, leaving behind only a stained streak or stripe which shows the same spiral curves as the original. This indicates that the saprospira, like many bacteria, has a viscid slimy or jelly-like membrane. This is further indicated by the way the organism sticks to the coverslip and remains there by any method of fixation or staining.

The free spores, however, are not retained in the preparations. The further development of the spores was not observed. Gross believes that they are true spores and in all probability develop and form new chambers.

Gross: Mitt. a. d. Zool. Stat. zu Neapel, 1911, xx, 188.

### SAPROSPIRA NANA Gross

Shorter and thinner than *Saprospira grandis*. Found also by Gross in foraminiferous sand. Survives in cultures three weeks, cultures consisting of material placed in a few drops of sea-water on slides. In the cultures at the high point of development thick clumps of the organisms occur approximated by their long axes and forming a kind of cue or pleat like those seen in the spironema.

Body colorless and wound in spirals. Longest individuals 36 $\mu$ long with 16 spiral turns. Thickness 0.5 $\mu$. Single spiral turns 2.25 to 3 $\mu$ long. Spirals open and turns not abrupt. No chambers made out in fresh material, but this may be due to the small size of the organism and its thick wall. With stained preparations the chambers can be made out. They are considerably longer than the chambers in *Saprospira grandis* and may occupy a half or a complete spiral turn. No definite division of chambers or pole caps observed.

Method of division not clearly established. The many short forms in young organisms indicate that the transverse division must take place more rapidly than in *Saprospira grandis*. In old cultures the chambers take on the characteristic light appearance seen in *Saprospira grandis* (empty chamber) and multiplication occurs according to only one type, the indentation of the chamber and its subsequent division from torsion. Since these empty chambers are very long after division, they may remain attached to one or both of the new daughter-cells as thin processes of considerable length. No spore-formation observed.

Gross: Mitt. a. d. Zoöl. Stat. zu Neapol, 1911, xx, 188.

### SAPROSPIRA FLEXUOSA Dobell

Described by Dobell in water from River Granta at Cambridge. Resembles *Saprospira grandis* of Gross. Found but once. Organism multiplied for about a month in laboratory cultures. Grew well in cultures containing a small amount of $H_2S$ as in case of fresh water spirochetes.

Colorless, motile, flexible organisms with a very regular, spiral form. Length and number of turns to spiral vary. Smallest individuals consist of less than one complete turn and are between 3 and 4 $\mu$ in length. Longest have maximum length of about 50 $\mu$ and consist of about sixteen turns. Breadth constant, about 0.8 $\mu$. Such measurements apply to living organisms. The cells are longer in fixed and stained preparations, due to stretching of spiral. In the living organism the diameter of the spiral is about 2 $\mu$, the distance between summit of one turn and that of next about 3 $\mu$. In fixed organisms the diameter of spiral tends to diminish, distance between turns to diminish. Ends rounded. No polar caps. No terminal flagella. Round in optical, transverse section. No crista or similar organ. Only terminal structure a number of very refractive granules. Living organisms colorless and transparent. No coloring-matter.

Movements consist of rotation, translation, and flexion, generally performed simultaneously. Rotation screw-like, resembling that of the spirilla, but executed more slowly. It always accompanies locomotion, which is never very rapid. No anteroposterior polarity.

Distance traveled in eighteen minutes about 250 $\mu$ in a short organism of about five turns. This traveled a distance of its own length in about one minute. The spiral itself remains constant during flexion. Mechanism of motility not made out. No crista. No terminal flagella. Ends smooth and round.

**Internal Structure.**—In living organism no definite protoplasmic structure. No clear indication of transverse septa dividing spiral into chambers. Only structures visible are refractive granules already mentioned. These stained red by intravital staining with neutral red or methylene-blue, and are metachromatic granules like those of other bacteria. They stain deeply by iron hematoxylin, but readily decolorize in differentiation.

Easily stained by good fixing agents, solutions of Bouin, Schaudinn, Flemming, Hermann, etc. Chambered like a cristispira from end-to-end. Protoplasmic chambers of fairly constant size, nearly as broad as long, about six in one complete turn of the spiral. They may be stained by Heidenhain, Delafield, borax carmin, safranin, methylene-blue. Transverse septa often very faint or invisible, never look like cell walls. Deeply stained individuals show no chambered structure which comes out by decolorization. Metachromatic granules lie in the walls of the chambers, *i. e.*, in the transverse protoplasmic septa as in pseudospira and other forms. No nuclear granules surrounding transverse septa made out.

Plasmolysis takes place in a peculiar way. Movement of organism ceases in 5 or 10 per cent. NaCl. The cell becomes slightly swollen and then suddenly develops blisters at different points along the spiral. The delicate and apparently elastic membrane surrounding the body swells up in the form of a globule, leaving the protoplasm on one side. Usually two blisters are formed, one at each end, with another in middle. In long individuals four to five may appear. The protoplasm in centrally-placed blisters frequently appears to disintegrate. This is the usual method of plasmolysis in Saprospira and *Pseudospira serpens*. It shows that the spiral is invested by an extensible membrane, very delicate, and tough enough to withstand considerable pressure from within without rupture. It also shows that the organism is not multicellular. If it were, each cell should plasmolyze separately as in cells of a spirogyra filament. Plasmolysis occurs in much the same way that a spirillum might plasmolyze if it possessed a very thin and extensible membrane, not a thick, rigid one. The thin, elastic membrane and the flexibility of the body are obviously correlated.

**Division** by multiple, transverse fission. It takes place in long spirals only, beginning as a regular series of breaks along the spiral. These breaks appear as pale transverse lines, one situated as a rule at the summit of each turn of the spiral or close to this point. Break first involves protoplasm only, not the membrane. Whole spiral thus becomes segmented into a number of short pieces, varying in length from about a half to one whole turn of the spiral. The spiral remains in this condition three or four hours. Actively motile. Subsequent changes seen in fixed and stained preparations. Later segmented individuals first connected by investing body membrane, this later being drawn out so as to form a delicate filament connecting two adjoining daughter individuals. These delicate connections subsequently break, setting free the young forms which frequently possess very darkly stained ends. Short daughter individuals, when first set free, contain from three to six protoplasmic chambers. They are short, comma-shaped, or slightly spiral organisms, moving very slowly or not at all. When longer they move more rapidly. Young forms resemble spirilla. Flexion most marked in longest spirals. Delicate threads derived from body membrane, at first connecting daughter individuals, are absorbed or dissolved. Never detected as flagellum-like processes at ends of completely separated young organisms. The multiplication is like that described by Gross for *Saprospira grandis*.

Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

### 4. GENUS SPIRONEMA Vuillemin

Type organism *Spironema recurrentis* Lebert.

Numerous pathogenic and non-pathogenic varieties. Usually blood parasites. Length 8 to 16 $\mu$. Pointed ends. Diameter 0.35 to 0.5 $\mu$. Cylindrical or slightly flattened. Large, wavy, inconstant spirals about five in number. Closer and more regular in cultures. Axial filament probably present. Delicate, flexible, double-contoured membrane. Spirals undulating up and down, not from side to side. Fine terminal, spiral, filaments. No chambered structure, crista, or highly motile end portion. Division transverse. Membrane difficult to differentiate. Body stains violet by Giemsa. Complete disintegration in bile salts (10 per cent.). Immobilization in saponin (10 per cent.) in thirty minutes, disintegration in a few hours. Axial filament laid bare in some species.

Schellack believes that *Spironema Obermeieri*, *Novyi*, and *Duttoni* are not true three-dimension spirals spirally wound, but two-dimension spirals, wave-like in a single plane.

*Man*

Type species *Spironema recurrentis* (Lebert).
" *Carteri* (Mackie).
" *Duttoni* (Breinl).
" *Kochi* (Schellack).
" *Berbera* (Sergent and Foley).
" *Egyptica* (Balfour).
" *Novyi* (Schellack).

*Birds*

*Spironema anserina* (Sakharoff).
" *Marchouxi* (Nuttall).
" *granulosa* (Balfour).
" *Nicollii* (Brumpt).
" *Neveuxi* (Brumpt).

*Pigs*

*Spironema suis* (King, Baeslock, and Hoffmann).

*Cattle*

*Spironema Theileri* (Laveran).
" *bovis caffris* (Nuttall).

*Horses*

*Spironema equi* (Novy and Knapp).

*Sheep*

*Spironema ovis* (Novy and Knapp).

*Monkeys*

*Spironema macaci* (Castellani and Chalmers).
" *pitheci* (Thiroux and Dufourgeré).

*Otter*

*Spironema lutræ* (Powazek).

*Guinea-pigs*

*Spironema caviæ* (Macfie).

*Bats*

*Spironema vespertilionis* (Novy and Knapp).
" *vesperuginis* (Gender).

*Serpents*

*Spironema tropidonoti* (Dobell).

*Rats*

*Spironema minor* (Carter).

*Mice*

*Spironema Laverani* (Breinl and Kinghorn).
" *muris* (Wenyon).
" *muris var. Virginiana* (MacNeal).

*Fish*

*Spironema Jonesii* (Dutton, Todd, and Tobey).
" *latapici* (Laveran).
" *gadi* (Neumann).
" *pelamidis* (Neumann),
" *pollackii* (Henry).

### SPIRONEMA RECURRENTIS (Lebert)

Originally observed by Obermeier[1] in relapsing fever in Eastern Europe and described as a spirillum. Named *Spirillum recurrentis* by Lebert in 1874. Described by Cohn[2] and by Koch.[3]

This organism is also the type of spironema found in relapsing fever in Russia and in Poland. During the period of pyrexia the characteristic spirals are easily demonstrated in the blood. Known also as *Spirochæta recurrentis*, *Spirochæta Obermeieri*, and *Spiroschaudinia recurrentis* or *Obermeieri*.

Fig. 178.—*Spironema recurrentis* from human blood, stained with carbolfuchsin.

**Morphology.**—Rather stout, spiral threads about 1 $\mu$ thick and 7 to 9 $\mu$ long with four to ten undulations. Longer forms may occur. Compared with the red blood-corpuscles the length of the organism may be one-half to nine to ten times the diameter of the corpuscles. Ends pointed. Often whirls or skeins of spiral threads are formed among the corpuscles. Migula states that the spirals are about 15 to 20 $\mu$ long and 0.4 $\mu$ thick. Besson describes very long organisms, 100 $\mu$ or more, which are occasionally seen, and says that the spirals tend to agglutinate and form rosettes in the blood. In the cultivated strains (Noguchi) young cultures contain very short motile forms showing only one to two curves, in addition to the active specimens of average length and width. They look unlike the forms seen in the blood of an infected mouse or rat which are quite regular. The cultivated forms are irregularly wavy and shorter. Pairs, chains, or large entangled masses occur. The cells have blunt ends with a mass of minute refractive granules attached. After degeneration two or more spherical bodies, measuring about 0.7 $\mu$ in diameter and resembling spores, are seen attached to many of the organisms.

Also free, spherical bodies and skeletons of spirals occur. Schellack[4] thinks that *recurrentis* is not a true three-dimension spiral, but is really wave-like in a single plane.

**Reproduction** by transverse fission. Two or more organisms often appear joined by a fine filament. The appearances originally interpreted as longitudinal division are now seen to be the twining together of separate individuals which gradually unwind.

**Motility.**—Active corkscrew-like motility in the blood, with lateral oscillations.

Novy and Knapp[5] have described single polar flagella and Norris, Pappenheimer, and Flournoy[6] have observed long, filamentous, tapering ends regarded as bipolar terminal flagella. These are interpreted by Breinl[7] as prolongations of the periplast. Zettnow[8] has described peritrichic flagella, but his observations have not been confirmed. Cultivated organisms (Noguchi) have a serpentine, forward movement with violent lateral vibrations involving the middle portions of the body and both ends.

**Stains** well in the blood by Loeffler's or Kühne's mtehylene-blue, carbolfuchsin, Romanowsky, or by Günther's method. The films should not be heated, but should be treated with very dilute aqueous acetic acid. Gram-negative. In tissues best demonstrated by Günther's method. Cell body homogeneous except in degenerated specimens, where it is granular.

**Cultivation.**—Organisms were kept alive in the original blood for forty days by Novy and Knapp, no definite multiplication being seen. Norris, Pappenheimer, and Flournoy obtained positive evidence of multiplication in fluid media by inoculating a few drops of spirochetal rat blood into citrated human or rat blood. Some multiplication in transfers from this blood, but none in the third generation. First definitely cultivated by Noguchi[9] in ascitic or hydrocele fluid containing a bit of sterile tissue (rabbit's kidney) at 37° C. Growth begins in twenty-four hours. Maximum growth on seventh day, after which the organisms degenerate. Under a layer of paraffin oil the organisms grow more slowly and remain active a day or two longer. Not filtrable through Berkefeld filters (Noguchi). No growth at room temperature. No growth "in vacuo" or in an atmosphere of hydrogen. For growth the spirochetes require fresh tissue and a body capable of forming loose fibrin from it. Pathogenicity retained in Noguchi's cultivated strains.

*Spironema Obermeieri* was also cultivated by Plotz[10] directly from patients with relapsing fever in Serbia by Noguchi's method. Finally it has been cultivated by Kligler and Robertson[11] on a basic medium of horse serum or rabbit serum diluted with 1 to 2 parts of undiluted ascitic fluid, with 1 per cent. peptone broth added and the reaction adjusted to *p*H 7.2. The medium is inoculated with 1 drop of infected blood or 0.1 c.c. culture and covered with oil 1.5 cm. high. In initial cultures the rat blood furnishes the fibrin. In subsequent cultures the fibrin is supplied by a drop of fresh rabbit blood. In place of fibrin 0.05 to 0.1 per cent. sugar may be used. Incubation at 28° to 30° C. After the culture is well started growth is good at room temperature. Viability retained three to seven weeks. Reaction limits for growth and survival, *p*H 6.8 and 8.2. Optimum *p*H 7.2 to 7.4. Kligler and Robertson believe that the organism is a strict aërobe and the layer of oil should not be more than 1.5 cm. high. Cultivated also by Aristowsky and Hoeltzer.[12]

**Virulence.**—*Spironema recurrentis* is virulent for mice and white rats; rabbits, guinea-pigs, and dogs being immune. It is also virulent to monkeys, especially the Cercopitheci. In monkeys subcutaneous inoculation is followed in two to four days by a pyrexia lasting several days, after which the temperature rapidly returns to normal. During the pyrexia the spirochetes are present in the blood. Occasionally two to three similar attacks may occur before immunity is established. In rats the incubation period is two to five days and the spirochetes are found in the blood in large numbers. Relapses may also occur. Nattan-Larrier,[13] who worked with Russian virus, finds that it penetrates the intact skin and mucous membrane of white rats. If the hairs be removed and a few drops of virulent defibrinated blood be dropped on, infection is produced in the regions where the skin is fine and vascular, as in the anterior region of the abdomen. The virus also passes through the mucous membrane of the mouth, rectum, vagina, and conjunctiva. It traverses the mucous membranes in several hours, the skin less rapidly. The period of incubation is longer than with subcutaneous incubation. It varies from three to five days for the vagina, rectum, and mouth, three to six days for the conjunctiva and genitalia of the male, three to seven days for the skin, average being seven days. Mortality low, 14 deaths in 43 cases compared with 6 deaths in controls. Duration of malady longer, crisis not so short, recurrences longer and more frequent, three to six occurring. Not so many spiral organisms appear in the blood. Nattan-Larrier believe that an attenuated infection is produced by this method of inoculation.

Insect transmission not definitely worked out. The organisms may live in bed-bugs (Zinsser). Killed at 60° C. Viability retained at 0° C.

Tomioka[14] states that after an attack of relapsing fever the parasites are present in the internal organs, especially in the brain. With large doses of salvarsan the spirochetes are destroyed and are but rarely found in the brain.

1. Obermeier: Centralbl. f. d. med. Wissensch., 1873, ii, 145.
2. Cohn: Beitr. z. Biol. d. Pflanz., 1875, i, Heft 3, 196.

3. Koch: Beitr. z. Biol. d. Pflanz., 1877, ii, Heft 3, 431.
4. Schellack: Arb. a. d. K. Gsndhtsamte, 1907, xxvii, 364.
5. Novy and Knapp: J. Infect. Dis., 1906, iii, 291.
6. Norris, Pappenheimer, and Flournoy: J. Infect. Dis., 1906, iii, 266.
7. Breinl: Ann. Trop. Med. and Parasitol., 1907–08, i, 435.
8. Zettnow: Deutsche med. Wchnschr., 1906, xxxii, 1, 376.
9. Noguchi: J. Exper. Med., 1912, xvi, 199.
10. Plotz: J. Exper. Med., 1917, xxvi, 37.
11. Kligler and Robertson: J. Exper. Med., 1922, xxxv, 303.
12. Aristowsky and Hoeltzer: Centralbl. f. Bakteriol. [etc.], 1925, xciv, 448.
13. Nattan-Larrier: Bull. Soc. path. exot., 1909, ii, 239.
14. Tomioka: Centralbl. f. Bakteriol. [etc.], 1924, xcii, 41.

### SPIRONEMA CARTERI (Mackie)

Described by Carter[1] in the blood of patients with Indian relapsing fever. Same as *Spirochæta Carteri* and *Spiroschaudinnia Carteri*. Called *Spirillum Carteri* by Mackie.[2] A similar spirochetosis in India has been reported by Turnbull.[3]

**Morphology.**—Measures 0.2 to 0.35 $\mu$ in diameter and 15 to 20 $\mu$ in length (Schellack[4]). Mackie,[5] who studied relapsing fever near Bombay, gives the length of the Bombay spirochete as 7 to 90 $\mu$. The ordinary length is 10 to 16 $\mu$, next most frequent 26 to 32 $\mu$. The increase in size is in multiples of ten to sixteen. Smaller spirochetes, 6 to 7 $\mu$ long, are seen, but this is not the rule. Width not more than 0.5 $\mu$, generally less. In fresh blood the organisms have a corkscrew shape. In films the curves are more open. The organisms tend to agglutinate or agglomerate in tangles or skeins. On the ends terminal filaments may be demonstrated by van Ermengem's or Loeffler's method. They gradually taper to a fine point and are not like true bacterial flagella. As the cells die the cytoplasm undergoes fragmentation and beading, the beads staining a deep red with Romanowsky like chromatin. Also deeply staining globular bodies appear, 1 to 2 $\mu$ in diameter. Division transverse. No undulating membrane.

**Motility** of three types—progressive, vibratory, and undulatory.

*Not cultivated.*

**Virulent** to white mice, white rats, white rabbits, black rats (*Mus rattus*), brown rats (*Mus decumanus*), guinea-pigs, and monkeys; not to goats or pigeons. Relapses occur only in monkeys. No deaths in susceptible animals. In relatively insusceptible animals the blood often shows tightly coiled forms looking like watch springs. Cohn[6] states that Carter produced spirillar fever in small East India apes, *Lemnopithecus entellus* and *Macacus radiatus*, by hypodermic injection of the defibrinated blood of man in the first recurrent attack. Fever developed on the sixth day and the blood was full of spirals, shorter, and with fewer turns than in human blood.

**Transmission.**—Mackie believes that transmission may be effected by *Pediculus corporis (vestimenti)*. Mackie found many cases of relapsing fever in boys who were heavily infested by *Pediculus corporis*, few cases in girls, who were lightly infested. Spirochetes were found in the mouth secretions and in large numbers in the stomach contents of the lice, also in the ovaries. Transmission of the disease to monkeys by *Pediculus corporis* was not demonstrated, but Mackie believes that the lice are the agents of transmission. *Pediculus capitis* and the bedbug, *Cimex lectularius*, are not so regarded.

This spirochete and Turnbull's organism are regarded by Novy and Knapp[7] as distinct from the spirochetes in other types of relapsing fever.

1. Carter: Deutsche med. Wchnschr., 1879, v, 351; 386.
2. Mackie: Lancet, 1907, ii, 832.
3. Turnbull: Indian Med. Gaz., 1905.
4. Schellack: Arb. a. d. K. Gsndhtsamte, 1907, xxvii, 364.
5. Mackie: Brit. M. J., 1907, ii, 1706.
———: N. York M. J., 1908, lviii, 337.
6. Cohn: Deutsche med. Wchnschr., 1879, v, 189.
7. Novy and Knapp: J. Infect. Dis., 1906, iii, 291.

### SPIRONEMA DUTTONI (Breinl)

Described by Dutton and Todd[1] in West African tick fever or West African relapsing fever. This affection is wide-spread in Africa as shown by the investigation of Ross and Milne,[2] Koch,[3] Dutton and Todd. The

spirochetes which cause the disease are different from those found in European relapsing fever, East African relapsing fever, etc. (Novy and Knapp,[4] Koch). Named *Spirochæta Duttoni* by Breinl.[5]

**Morphology.**—As described by Dutton and Todd the organisms are 13 to 43 $\mu$ long with an average length of the long forms of 35 $\mu$, of the short forms 16 $\mu$. Arranged in twos, threes, fours, and Y-shaped forms. Width 0.45 to 0.5 $\mu$.

**Division Transverse.**—In spirochetes from infected monkeys stained by Giemsa's modification of Romanowsky's stain Breinl[6] has described a centrally stained core surrounded by a lightly stained periplast. This sheath is drawn out into an elongated filament described by others as a terminal flagellum. The central core (chromatic) does not stain uniformly, but shows darker and lighter areas. This chromatic core is frequently broken up into successive granules. The organisms also show one or more central or peripheral swellings and a small unstained transverse band about a third from the end. No undulating membrane. In the liver, rarely in the spleen and bone-marrow, the spirochetes coil up, swell, become thinner, and roll up into a complicated skein just before the crisis. The organisms are phagocyted by cells of the spleen and at time of the crisis the spleen cells are gorged with degenerated spirochetes. In animals with spleen removed analogous phenomena may be observed in the liver. These skein-like parasites are surrounded by a thin cyst wall with faintly stained blue plasma inside. Later only small, red, granules appear inside the cysts. Breinl thinks that these granules may be infectious. Schellack[7] regards this organism as not a true three-dimension spiral, but really wave-like in a single plane.

In young cultures short forms are numerous (Noguchi). At the period of maximum growth the length is about that in normal infections. Curves regular and deep. Organisms single, in pairs, or chains. Vigorously motile. In old cultures the organisms lose their motility, degenerate, and protoplasmic masses concentrate at various points along the skelcton of the spirals. Many parasites show one or more spherical bodies attached laterally at various intervals.

**Motility** active in the blood and in young cultures. Motility lost in old cultures. Peritrichic flagella have been described by Zettnow, but this has not been confirmed.

**Stains** best by Romanowsky or by Giemsa's modification of Romanowsky.

**Cultivated** first by Levaditi in the serum of *Macacus*, heated to 70° C., in collodion sacs in the peritoneal cavity of rabbits. An Indian spirochete was also cultivated by Novy and Knapp in non-coagulated rat blood in collodion sacs for twenty generations. Cultures scanty. Virulence for rats retained seven days. Cultivated by Noguchi[8] in ascitic or hydrocele fluid in 15 c.c. quantities containing a small piece of sterile tissue (rabbit's kidney), inoculated with a few drops of citrated blood from an infected animal. The medium may be covered with paraffin oil or be left uncovered. The organisms show multiplication in forty-eight hours and are decidedly increased in three days. Maximum growth on eighth to ninth day. Diminution on the tenth day. The spirochetes multiply more slowly and disappear later when the tubes are covered with paraffin oil. No multiplication and prompt degeneration at 15° C. No growth in an atmosphere of hydrogen or "in vacuo." The organisms require fresh tissue and loose fibrin for growth. No visible changes take place in the culture-media. No coagulation. No odor. The tissue may look somewhat paler. Pathogenicity for mice and rats retained in cultures.

Not filtrable through Berkefeld filters (Noguchi).

**Pathogenic** for mice, rats, rabbits, and monkeys. Breinl and Besson[9] find that *Spironema Duttoni* is more virulent for lower animals than *Spironema recurrentis*. Monkeys, Cercopithici and Macacus, are very susceptible. Mice and rats die from intraperitoneal inoculation of human blood. Successive attacks develop over a period of three to forty-five days with a hypertrophy of the spleen and hemorrhages into the organ. Rabbits have severe attacks from large doses (5 c.c. or more) given intraperitoneally. Guinea-pigs are less susceptible. Breinl and Kinghorn have produced a mild infection in rats from blood diluted 1 : 10 and filtered through a Berkefeld filter.

Transmission by the tick, *Ornithodorus moubata*, as shown first by Ross and Milne and by Dutton and Todd. Also by the bites of young ticks newly hatched in the laboratory from infected parents. Koch and Carter[10] have found spirochetes in the eggs laid by infected female ticks. Experimental infection may be produced in monkeys by allowing infected ticks to bite them. The spirochetes undergo profound changes in the body of the ticks, as pointed out first by Dutton and Todd, later by Leishman.[11] Lateral or terminal swellings containing one or more particles of chromatin develop with a fragmentation of the chromatic core of the spirochete into numerous small coccoid or bacillary bodies. Leishman found no recognizable spirochetes in the tick after the tenth day, but small chromatin particles free in the gut and aggregates of granular clumps in the Malphigian tubes and ovaries, rarely in the salivary glands. At this stage the ticks are still capable of producing infection. Leishman regards these chromatin granules as derivatives of the spirochetes and considers that they are infectious particles, a stage in the life history in the tick. Dutton and Todd state

that spirochetes can always be found in infected ticks if the search for them be sufficiently prolonged. A definite transformation of granules into spirochetes has not thus far been observed. Brumpt[12] reports that *Duttoni* is also transmitted by *Ornithodorus Savigni*, which closely resembles *Ornithodorus moubata*. *Spironema Duttoni* differs from the other spironemata of relapsing fever immunologically. In animals it conveys immunity to itself, but not to other spirochetes.

1. Dutton and Todd: Brit. M. J., 1905, ii, 1259.
2. Ross and Milne: Brit. M. J., 1904, ii, 1453.
3. Koch: Deutsche med. Wchnschr., 1905, xxxi, 1865.
4. Novy and Knapp: J. Infect. Dis., 1906, iii.
5. Breinl: Lancet, 1906, ii, 1690.
6. Breinl: Ann. Trop. M. and Parasitol., 1907–08, i, 435.
7. Schellack: Arb. a. d. K. Gsndhtsamte, 1907, xxvii, 364.
8. Noguchi: J. Exper. M., 1912, xvi, 199.
9. Besson: p. 713.
10. Carter: Ann. Trop. Med. and Parasitol., 1907–08, i, 157.
11. Leishman: Lancet, 1910, i, 11.
12. Brumpt: Bull. Soc. path. exot., 1908, i, 432.

### Abyssinian Spirochetosis (Tick fever)

Spirochetosis has been described by Brumpt in Abyssinia. Brumpt allowed *Ornithodorus moubata* to feed on *Macacus rhesus* and produced a relapsing fever with spirochetes in the blood. Rats bitten by ticks did not develop infection. This Abyssinian spirochete was identical morphologically with *Spironema Duttoni*, but was regarded by Brumpt as a variety which gives relatively attenuated reactions in animals. The Abyssinian tick fever is possibly transmitted by *Ornithodorus moubata*.

Brumpt: Bull. Soc. path. exot., 1908, i, 432.

### Spironema Kochi (Schellack)

Spirochetes in East African relapsing fever were originally observed by Koch and named *Spirochæta Kochi* by Schellack,[1] *Spirochæta Rossi* by Besson,[2] and *Spiroschaudinnia Rossi* by Nuttall and by Castellani and Chalmers.[3]

**Morphology.**—In cultures the organisms are a little thinner than *Spironema Duttoni*. They show round bodies along the periplast.

Division transverse.

**Cultivated** by Noguchi[4] in ascitic or hydrocele fluid containing a bit of fresh tissue like rabbit's kidney. Maximum growth on the ninth day. No visible alteration of media. No odor. Growth aërobic, takes place at 37° C., but not at room temperature. No growth in an atmosphere of hydrogen or in vacuo. The organisms require tissue and a body forming loose fibrin from the tissue. Pathogenicity gradually attenuated. Not filtrable through Berkefeld filters (Noguchi). A spirochete from African relapsing fever was also cultivated by Hata,[5] who used a mouse-to-mouse strain and employed a semicoagulated mass of normal horse serum, to which twice its volume of physiological salt solution was added with a piece of rabbit's kidney. Growth begins in twenty-four to forty-eight hours. Maximum growth on fourth day. Organisms often agglutinated into tangled masses. The buffy coat of horse serum consisting of white corpuscles and blood-platelets may be substituted for the kidney. It is cut into pieces 1 cm. square and two to three are used in each tube. Here growth begins on the second day and reaches its maximum in five to seven days. By the first method the virulence is relatively weak, small doses of cultures two to four days old causing only a mild infection. From cultures five to seven days old no infection occurs in mice. Virulence retained better by the second method. Good in cultures five to seven days old, less in cultures ten days old, retained up to twenty-one days. It is not entirely clear whether Hata's strain represents *Spironema Duttoni* or *Spironema Kochi*.

1. Schellack: Arb. a. d. K. Gsndhtsamte, 1907, xxvii, 364.
2. Besson: p. 712.
3. Castellani and Chalmers: Manual of Tropical Medicine. London, 1919, p. 445.
4. Noguchi: J. Exper. M., 1912, xvi, 199.
5. Hata: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1914, lxxii, Orig., 107.

### Japanese spirochetosis

A spirochete has been cultivated by Hata from relapsing fever in Tokyo which is apparently distinct from the spirochetes found in African relapsing fever.

Hata: Centralbl. f. Bakteriol. [etc.], 1 Abt., 1914, lxxii, Orig., 107.

### Arabian spirochetosis

Described by Carter in the Mudariba district of Arabia and in camps in Senawi. The type of fever resembles that found in Africa due to *Spironema Duttoni*. The camps were infested with ticks belonging to the Ornithodorus group.

In man the spirochetes in the blood were single or in pairs, joined end-to-end by fine cytoplasmic bands. Ends pointed. Stain faintly. Organisms of two types, one with scanty wide and irregular plication, another with regular, shallow, and more numerous plication. Peculiar bodies were described by Carter, globose central thickenings consisting of a cytoplasmic sheath and enclosing large, oval bodies staining deep purple, to which a more faintly staining mass was connected, bipolar, oval bodies staining deep carmin at each end, pyriform deeply staining central bodies with no definite cytoplasmic sheath, and oval, cytoplasmic, central vesicles enclosing two oval bodies staining by carmin.

Human blood from 2 cases was injected intraperitoneally in young bush-tailed rats (Gerbun). These animals developed spirochetosis with enlarged spleen. No infection produced in coneys, chameleons, or spiny-tailed lizards.

Carter: Indian Med. Gaz., 1908, xliii, 370.

### Spironema Berbera (Sergent and Foley)

Described originally by Sergent and Foley[1] as *Spirochæta Berbera* and found by them in recurrent fever epidemic in Algiers. Same as *Spiroschaudinnia Berbera.*

**Morphology.**—In the blood of man or monkeys the organism appears as a very fine, refractile spiral with pointed ends. Granular or vacuolated in unstained preparations. When stained by Giemsa the length varies from 12 to 24 $\mu$, average thickness is 0.25 to 0.3 $\mu$, number of spirals three to nine. Length of a complete spiral or turn is 2.5 to 3 $\mu$. Width of spiral 1 to 1.4 $\mu$. Division transverse, the resulting organisms sometimes being connected by fine filaments difficult to stain. No chains of more than two elements. No Y-shaped branching suggestive of longitudinal division.

**Motility** active, boring, undulatory, and progressive in either direction.

*Not cultivated.*

**Pathogenic Action.**—Taken directly from man this organism is virulent for monkeys, *Macacus cynomolgus*, *Macacus innus*, *Macacus sinansi*, and *Cynocephalus sphinx*. The animals, after an incubation period of less than forty-eight hours, die in five to six days after subcutaneous and intraperitoneal inoculation. The spirochete also produces light non-fatal infections in rats and mice. The blood of monkeys which succumb to infection will not produce disease in other monkeys. Monkeys which have been inoculated with the virus of European relapsing fever (Russian virus) are not resistant to the Algerian virus. The spirochetes from the Algerian disease are not agglutinated by immune serum from animals recovering from the European virus and vice versa.

**Transmission** by the louse, *Pediculus vestimenti*, suggested by Sergent and Foley[2] and determined by Sergent, Gillot, and Foley[3] in an epidemic at Sud-Oranais. Disease was produced in monkeys by subcutaneous inoculation of infected lice. Incubation period six to eight days like that in the natural disease. Sergent and Foley[4] have further shown that in the lice the spirochetes undergo a latent period in their development. For eight days after feeding on infected blood the lice contain the virus despite the fact that spirochetes cannot be seen. They appear later. Sergent and Foley conclude that the spirochetes take on a small form in the period of apyrexia in man and in the body of the louse, a kind of granular stage. According to Brumpt[5] no transmission occurs by *Ornithodorus moubata*.

In the treatment of the disease good results were obtained with arsenobenzol.

1. Sergent and Foley: Ann. de l'Inst. Pasteur, 1910, xxiv, 337.
2. Sergent and Foley: Bull. Soc. path. exot., 1908, i, 174.

3. Sergent, Gillot, and Foley: Compt. rend. Soc. de biol., 1911, lxx, 1039.
4. Sergent and Foley: Compt. rend. Acad. de sc., 1914, clxix, 119.
5. Brumpt: Bull. Soc. path. exot., 1908, i, 432.

### Spironema Egyptica (Balfour)

Egyptian recurrent fever was reported by Bonsfield[1] and the spirochetes in the disease described by Balfour.[2]

The organisms are 13.5 to 22.5 $\mu$ in length and 0.25 $\mu$ in width, with two to six spirals. They show great irregularity in arrangement, loops, figures of eight, circles, etc. The infection may be transmitted to monkeys, *Cercopithecus sabaceus*, and to desert mice, *Gerbillus pygargus*. The monkeys develop fever, but do not die, the spirochetes being found in the blood. They are also present in the blood of the gerbils for a short time. Organisms not cultivated. Transmission not worked out, but the disease is apparently not carried by ticks (Dreyer[3]). It may be the same as the Algerian relapsing fever. Thèzè[4] reports a case from Madagascar and Cristopher[5] cases from the Sudan.

1. Bonsfield: J. Roy. Army Med. Corps, 1910, xv, 444.
———: Fourth Rep. Wellcome Tropical Research Lab., Khartoum, 1911, A, 62.
2. Balfour: J. Roy. Army Med. Corps, 1910, xv.
———: Fourth Rep. Wellcome Tropical Research Lab., Khartoum, 1911, A, 67.
3. Dreyer: Arch. f. Schiffs-u Tropen-Hyg., 1910, xiv, 37.
4. Thèzè: Bull. Soc. path. exot., 1911, iv, 509.
5. Cristopher: J. Trop. Med. and Hyg., 1909, xii, 353.

### Spironema Novyi (Schellack)

Discovered in American relapsing fever by Norris, Pappenheimer, and Flournoy,[1] and named *Spirochæta Novyi* by Schellack.[2]

**Morphology.**—Thinner than *Spironema recurrentis*. Schellack regards this spirochete as not a true three-dimension spiral spirally wound, but really wave-like in a single plane. It probably multiplies by transverse fission. Round bodies along the periplast were observed by Noguchi.

**Multiplication** in citrate blood at 37° C. was noted by Norris, Pappenheimer, and Flournoy. Cultivated by Noguchi[3] in ascitic or hydrocele fluid containing a piece of sterile tissue (rabbit's kidney) at 37° C. No growth at room temperature. Organisms aërobic, not growing in an atmosphere of hydrogen or "in vacuo." They require fresh tissue and a substance forming loose fibrin from it. The virulence of Noguchi's organism was retained in cultures.

Not filtrable through Berkefeld filters (Noguchi).

Darling[4] has reported cases of American relapsing fever from Panama with the isolation of similar organism. The spirochetes were actively motile with a rotatory and undulatory movement and underwent spontaneous agglutination. They consisted of rigid spirals staining homogeneously, appearing purple with polychrome blues. Gram-negative. Single spirochetes measure 7.2 to 13.2 $\mu$, double 13.2 to 17 $\mu$. Virulent to white mice, white rats, and monkeys. Darling made out two strains, A and B, with different immunological reactions.

1. Norris, Pappenheimer, and Flournoy: J. Infect. Dis., 1906, iii, 267.
2. Schellack: Arb. a. d. K. Gsndhtsamte, 1907, xxvii, 199; 364.
3. Noguchi: J. Exper. M., 1912, xvi, 208.
4. Darling: Arch. Int. Med., 1909, iv, 150.
*See also* Novy and Knapp: J. Infect. Dis., 1906, iii, 291.

### Recurrent Fever in Colombia

Robledo has described a type of relapsing fever in Colombia due to a spirochete. The organism is actively motile, has 6 to 10 coils, and shows clear spaces inside like vacuoles. Stains by Giemsa. The symptoms are like African tick fever. Robledo believes that the infection is carried by *Argas americanus* (*Ornithodorus chinche*), the chinche bug, and concludes that the parasite of the disease does not differ from *Spironema duttoni*.

Robledo: Bull. Soc. path. exot., 1909, ii, 117.

### Spironema anserina (Sakharoff)

Observed originally by Sakharoff[1] in a septicemia occurring among geese in Tiflis and described as *Spirochæta anserina*. The infected birds become apathetic, stop eating, do not move, and die in about a week. Temperature 42.5° to 43° C. They show diarrhea and tenderness in the joints of the feet. At autopsy the body is emaciated, the spleen soft, and there is a fatty degeneration in the liver and heart muscle. Investigated especially by Dschunkowsky and Luhs.[2]

**Morphology.**—The spirochetes are like *Spironema recurrentis* except that the spirals are rigid. Length and number of spirals variable. Length 6.86 to 15.01 μ. Width 0.1 μ. Spirals 2.7 μ. Length of spiral 2 to 3.05 μ. Width 0.28 to 1.43 μ. When stained with gentian-violet 3 to 5 clear transverse lines appear in the body of the organism. At the beginning of the illness the typical spirochetes are found in the blood. Later, as the animals are about to die, the organisms form balls or clumps with a central mass of spirochetes and a lace-like periphery. They diminish in number and then disappear.

**Motility** like that of *Spironema Obermeieri* (*recurrentis*). The organisms move without turning about their long axis, the spirals remaining immovable. It is believed by Sakharoff that the organisms have terminal flagella at both ends. Not cultivated by Sakharoff. A multiplication observed by Dschunkowsky and Luhs in citrated goose blood plasma, in the abdominal cavity of rabbits. The transparent liquid contained very minute masses of soft tufts which formed a white deposit composed of spirochetes of various sizes. They were swollen in the center and at the extremities. In the fifth generation they divided into motile corpuscles like the infectious agent of pneumonia in cattle.

**Pathogenic Action.**—By the inoculation of small quantities of infected blood (1 drop) Sakharoff produced the disease in normal animals. They became sick in four to five days with spirochetes in the blood. Dschunkowsky and Luhs carried the virus along in geese. The first 25 died in nine days, later geese died in five days, at which time the parasites were most abundant in the blood. In geese living more than six days necrotic foci appeared in the liver and spleen. Disease of a mild character transmitted also to turkeys, canaries, crows, magpies, sparrows, and larks. Not transferred to pigeons.

Dschunkowsky and Luhs have found that atoxyl is a specific agent in this disease. One injection of 0.3 to 0.5 gram of atoxyl, two hours before artificial infection or at the same time, prevents the development of the disease. Two doses, 0.3 to 0.4 gram, twenty-four hours apart even on the third or fourth day save geese, although many spirochetes may be in the blood. A lasting immunity is conferred. A vaccine was produced by injecting a large dose, 20 to 40 grams of virulent blood, into a goose treated with atoxyl or vaccine. The serum of this goose was used for immunization. One injection, 0.01 gram of this serum, given with the virus prevented the development of the disease, 5 grams cured geese already infected.

1. Sakharoff: Ann. de l'Inst. Pasteur, 1891, v, 564.
2. Dschunkowsky and Luhs: Rep. Trop. Sec. Internat. Vet. Cong. at Hague, September 13–19, 1909. J. Trop. M. and Hyg., 1909, xii, 292.

### Spironema Marchouxi (Nuttall)

Described originally by Marchoux and Salimbeni[1] in hens in Rio de Janeiro. The infected fowls are somnolent, do not take nourishment and have diarrhea. They die in spasms after three to four days. A chronic form develops with death in eight to fifteen days. Temperature 42° to 43° C. in height of illness, falls to 41° C., and back to normal if the animal recovers. Temperature subnormal before death in fatal cases. At autopsy the spleen is enlarged to three times its normal size, the liver shows fatty degeneration with focal necrosis. Spirochetes are present in great number in the blood at the height of the illness. Named *Spirochæta Marchouxi* by Nuttall[2] in 1904 and *Spirochæta gallinarum* by Stephens and Cristopher in 1905. Known also as *Spirochæta gallinæ*. A similar or the same disease described in Senegal, India, by Craig, in Cairo by Bitter, in South Australia by Johnson and Nuttall, in Cyprus by Williamson,[3] in Samoli by Blaizot.[4] Brumpt and Foley[5] have noted a spirochetosis of fowls in South Algeria. The parasites were found in the blood and the infection transmitted by ticks. Immunity

experiments indicated that this virus was the same as the Brazilian virus of Marchoux and Salimbeni. Gerlach[6] has recently called attention to the prevalence of fowl spirochetosis in Austria.

**Morphology.**—In the blood the spirochetes occur singly or in small loose masses or groups. They measure 10 to 12 $\mu$ in length and 0.25 $\mu$ in width (Novy). In Noguchi's[7] cultivated organism he found the length 8 to 16 $\mu$, width 0.3 $\mu$, and chains of 2, 3, or 4 organisms. Parasites joined at times by a thin filament 2 $\mu$ long. Distance between curves 1.8 $\mu$. Average depth 1 $\mu$. Apex of curve usually round. Position of curve changes in violent movement. Round, refractile bodies, attached to the periplast along the side and usually single, were observed by Noguchi. These may represent a phase in the life cycle. Round or ovoid, highly refractile granules appear in old cultures, 0.2 to 0.3 $\mu$ in diameter, and may show active molecular movements. They are derived from the spirochetes and may be observed to leave it, empty immobile skeletal axial filaments only remaining. Such bodies are degeneration granules and fragments of the periplast. Division transverse by strangulation.

**Motility** very active. The organisms advance rapidly by turning about the long axis with corkscrew movements, the body remaining rigid. Also a lateral vibration occurs, the body forming a ring first in one direction, then in another. Besson[8] states that the flagella may be stained by a mordant of tannate of iron and fuchsin. They are long, visible all over the body of the parasite, and especially at one end, the other end being free of cilia. The short forms have only terminal tufts of flagella. Some organisms seem to have a long flagellum at one or both ends. Noguchi finds that the cultivated spirochetes have delicate finely curved projections attached to one end, about 3 $\mu$ long, rarely at both ends. These projections may be devoid of curves. They are prolongations of the periplast and continuous with the axial filament.

**Staining Reactions.**—Stained by dilute carbolfuchsin or Giemsa.

**Cultivation.**—Kept alive for one generation by Borrel and Burnet in citrated fowl blood or serum obtained by defibrination and centrifugation. Cultivated by Levaditi in fowl serum in celloidin capsules in the peritoneal cavity of guinea-pigs. First cultivated outside the body by Noguchi in his special medium of ascitic fluid broth containing a bit of fresh tissue covered with paraffin oil. Growth appears in twenty-four hours at 37° C., reaches its maximum on the fifth day, then recedes. No change occurs in the culture-media except a faint opalescence. No odor. No growth at room temperature or "in vacuo" at 37° C. Cultivated organisms retain their virulence. Not filtrable through Berkefeld V.

**Killed** easily by heating or by keeping at low temperature.

**Pathogenic Action.**—The virus is easily transferred from chicken to chicken, to geese, ducks, pigeons, sparrows, and turtle doves. Infection can be produced by the inoculation of infected blood, by feeding infected blood, or the dejecta of sick animals. The organisms, outside the animal body in the blood and juice of organs and in the dejecta, lose their virulence in forty-eight hours. Levaditi and Manouelian[9] have found that *Spironema gallinarum* does not proliferate exclusively in the vascular system. The parasite invades the glandular tissues and comes in contact with the different cellular elements. It does not seem to penetrate the protoplasm of the cells. Crisis is due to a phagocytosis of the organisms by the makrophages of the spleen and liver. The organism may also infect the ovule. One attack confers immunity and animals may be immunized by blood containing the organisms kept two to four days or heated to 55° C. for ten minutes. Serum from recently recovered animals filtered through Berkefeld filters will also immunize animals. It agglutinates the organism "in vitro."

Levaditi[10] states that the Brazilian fowl spirillosis may be transmitted to rabbits. Brumpt and Foley's virus in South Algeria was virulent to fowl, geese, ducks, pigeons, white owls, and "paddas" (Java sparrows).

Transmission by ticks, *Argas persicus*, under natural conditions was first shown by Mardoux and Salimbeni, and by *Argas miniatus*, *Argas reflexus*, and *Ornithodorus moubata* in laboratory experiments. The ticks may harbor the parasites for long periods, five months. In the fowl spirillosis in Cyprus described by Williamson, the fowls were heavily infested by *Argas reflexus*. Blaizot, working with fowl spirochetosis in Somali, found that the virus could be transmitted to young chicks by *Argas persicus* and that it increased in strength after passage. Fowls inoculated with chick virus and recovering were sensitive to the fowl virus. Blaizot concludes that the adult virus and the chick virus are different.

**Life Cycle.**—Hindle[11] has shown that *Spironema gallinarum* consists of a thick cell-wall, the periplast, surrounding a homogeneous core which stains like chromatin. It has no nucleus and no chromatic granules. Large cyst-like bodies occur which are degeneration forms. Coccoid bodies develop like those described by Balfour[12] in *Spironema granulosa penetrans*, and by Bosanquet in *Cristispira anodonta*. These are not true bacterial spores, since they multiply and stain deeply. Their development into spirochetes was followed in the body of the tick, not in the blood of infected fowls. In the tick the spirochetes pass through

the wall of the gut into the celomic fluid in two to forty-eight hours. They then bore their way into the salivary glands and the gonads of the tick. Here they often coil up, produce cyst-like forms and break up into coccoid bodies which multiply by transverse fission, especially in the cells of the Malphigian tubules and ovary. Coccoid bodies are also formed in the gut of the tick. If the infected ticks are kept at 37° C. for about five days spirochetes appear in the celomic fluid, in the lumen of the gut, and in all the organs. By making films from unincubated ticks and those kept various periods at 37° C. Hindle has observed all stages of development from coccoid bodies to spirochetes. The actual growth of coccoid bodies to spirochetes was not followed in living material, and the inoculation of coccoid bodies into Java sparrows did not produce infection. Hindle's observations on the life cycle of *Spironema gallinarum* have not been confirmed. No evidence has thus far been presented that in culture-media coccoid bodies develop into spirochetes.

1. Marchoux and Salimbeni: Ann. de l'Inst. Pasteur, 1903, xvii, 569.
2. Nuttall: Epidemiol. Soc., London, xxiv, 12.
3. Williamson: J Trop. M. and Hyg., 1909, xii, 271.
4. Blaizot: Compt. rend. Soc. de biol., 1909, lxvii, 421; 447. *Ibid.*, 1910, lxviii, 29.
5. Brumpt and Foley: Compt. rend. Soc. de biol., 1908, lxv, 132.
6. Gerlach: Centralbl. f. Bakteriol. [etc.], 1924, xciv, 84.
7. Noguchi: J. Exper. M., 1912, xvi, 620.
8. Besson: p. 718.
9. Levaditi and Manouelian: Ann. de l'Inst. Pasteur, 1906, xx, 593.
10. Levaditi: Ann. de l'Inst. Pasteur, 1904, xviii, 129. Levaditi and Lange: Compt. rend. Soc. de biol., 1905, lvii, 843.
11. Hindle: Parasitology, 1911, iv, 463.
12. Balfour: J. Trop. M. and Hyg., 1911, xiv, 113.

### SPIRONEMA GRANULOSA (Balfour)

Described by Balfour[1] in the spirochetosis of fowls in the Sudan and named by him[2] because of the life cycle of the parasite, a stage of which he believes to take place inside the erythrocytes. The disease in the Sudan is clinically like the Brazilian fowl spirochetosis and spiral organisms are present in the peripheral blood in large numbers. It may be very light and the spirochetes present in such small numbers that they are difficult to find. A similar spirochetosis was observed by Balfour[3] in geese north of Khartoum and traced to the same parasite. Bouet[4] has likewise recorded spirochetosis in fowls in Haut-Sénégal-Niger like Brazilian spirochetosis. This disease shows recurrences not seen in the Brazilian infection.

**Morphology.**—Short forms with four spirals 9 $\mu$ in length occur as well as medium forms with five spirals, 12 $\mu$ in length, fairly long organisms with eight spirals 16.5 $\mu$ in length, and extra long examples with nine spirals, 19.5 $\mu$ long. When stained by Giemsa or Leishman after fixation in absolute alcohol the organisms show diffuse nucleation, a central nuclear core surrounded by periplast, with chromatic dots in this nuclear helix. Ends pointed.

**Motility** active.

Transmitted by *Argas perspicus*. Incubation period forty-eight hours.

**Pathogenic Action.**—Virulent blood of fowls produces the disease in normal fowls in three days. Disease also transmitted to geese by inoculation, but not to pigeons. After one attack an immunity to new infection develops. According to Bouet fowls recovered from experimental Sudanese spirochetosis resist infection with Brazilian virus and with Senegal virus. He regards the Sudanese, Senegal, and Brazilian spirochetes as the same.

**Life Cycle.**—Balfour finds that animals, after the acute stage of the disease, develop a subacute or chronic stage which he characterizes as an "after phase." At this time curious intracorpuscular bodies appear in the erythrocytes due to invasion by the spirochetes. These bodies break up "in vivo" and "in vitro" into large granules which Balfour believes may be a stage in the life cycle of the parasite. In young chicks intracorpuscular bodies are rapidly motile and exhibit tiny motile filaments at the center. Relapses are common in chicks. The spirochetes disappear from the blood, then the "after phase" supervenes, the intracorpuscular bodies appear, and while they are still present the spirochetes recur. In stained preparations from the blood both spirochetes and intracorpuscular bodies may be found.

In ticks, *Argas persicus*, the larva and nymphs of adults fed on chicks with acute spirochetosis exhibit the peculiar chromatin granules first described by Leishman[5] in *Ornithodorus moubata* infected by *Spironema Duttoni*. They are present in ovaries, eggs, evoducts, alimentary diverticula and salivary glands of the ticks, but in greatest number in the Mal-

pighian tubules. Only granules penetrate the cells, not the spirochetes. Chicks inoculated with crushed larvæ, showing granules but no spirochetes, and with tissues or eggs of ticks containing granules, develop acute spirochetosis. Both spirochetes and granules appear in the blood of infected animals.

Balfour's granules have been observed by Bouet in the spirochetosis described by him. Often after the crisis spirochetes are rarely found, and at this time the granules may be observed in the red blood-corpuscles of the peripheral blood and in the bone-marrow. Hindle[6] believes that Balfour's intracorpuscular bodies are not nuclear degenerations and are not observed in Brazilian spirochetsois. They seem to represent a distinct phase of development.

No satisfactory explanation of Balfour's observations has ever been presented. The bodies described by Balfour have never been observed to develop into spirochetes. They may be stages of other parasites with which the Sudanese fowls were infected at the same time.

1. Balfour: J. Trop. M. and Hyg., 1907, x, 153. *Also:* Brit. M. J. 1907, ii, 1330.
2. Balfour: J. Trop. M. and Hyg., 1911, xvi, 113.
3. Balfour: Rep. Wellcome Trop. Research Lab., 1908, iii, 38. Parasitology, 1912–13, v, 122.
4. Bouet: Bull. Soc. path. exot., 1909, ii, 288.
5. Leishman: J. Roy. Army Med. Corps, 1909, xiii, 123.
6. Hindle: Parasitology, 1912–13, v, 127.

### Spironema Nicollii (Brumpt)

Spirochetosis in geese in Tunisia was first described by Duclaux and Nicolle,[1] subsequently by Comte and Bouquet,[2] and by Galli-Valerio.[3] The organism studied by Galli-Valerio was named *Spirochæta Nicollii* by Brumpt.[4]

The organisms are found in the blood and are refractile with tapering ends. The undulations are larger than those of *Spironema Obermeieri* and vary from 10 to 25. Length varies from 10 to 20 $\mu$ (Galli-Valerio). Motility rapid, rotatory, and progressive. It persists four hours under the microscope. The organisms are spontaneously agglutinated in the blood. Stained by dilute aqueous carbolfuchsin (1 : 1), Leishman and Giemsa (1 : 10). Transmitted by *Argas persicus*.

Disease conveyed by feeding infected *Argas persicus* to chickens by Galli-Valerio. Transmitted to ducks by Duclaux and Nicolle. Incubation period five to six days after tick bite, one and a half to two days after blood inoculation.

Balfour's intracellular corpuscles found in the disease by Galli-Valerio.

1. Duclaux and Nicolle: Rev. vét., 1903, 695. Arch. de l'Inst. Pasteur de Tunis, 1906, p. 39.
2. Comte and Bouquet: Arch. de l'Inst. Pasteur de Tunis, 1908, iv, 163.
3. Galli-Valerio: Centralbl. f. Bakteriol. [etc.], 1908, xlii, 495. *Ibid.*, 1909, l, 189. *Ibid.*, 1912, lxi, 529.
4. Brumpt: Prècis de parasitologie, Paris, 1910.

### Spironema Neveuxi (Brumpt)

Spirochetes were found by Brumpt in a fowl spirochetosis produced by the inoculation of *Argas persicus* collected by Dr. Neveux in Senegal and named *Spirochæta Neveuxi* by Brumpt.

The organisms are almost identical morphologically with *Spironema gallinarum*. Young forms have three spiral turns and are 8 to 10 $\mu$ long. Forms about to divide have six to seven spirals and measure 15 to 19 $\mu$ in length. Undulations narrower than in *gallinarum*. Virulent to fowls, geese, ducks, mice, and Javanese sparrows. Not to sparrows or finches. Pigeons not tried. Less virulent to fowls than *gallinarum*. Brumpt[1] has found that fowls recovered from *Spironema gallinarum* contract spirochetosis due to *Spironema Neveuxi*, and fowls immunized to *Spironema Neveuxi* are not immune to *gallinarum*. The Senegal virus is thus different from *gallinarum*. Fowls recovered from *Spironema gallinarum* from South Algeria and from Somali are sensitive to *Spironema Neveuxi*. The Senegal virus is thus different from the South Algerian and the Somail virus. Bouet[2] believes that the Senegal virus, his Sudanese, and the Brazilian are identical. They are different from another Senegal virus from Thies. On the basis of further experimentation Brumpt[3] has concluded that the Senegal virus is the same as the Brazilian.

1. Brumpt: Bull. Soc. path. exot., 1909, ii, 285. Prècis de parasitilogie. Paris, 1910.
2. Bouet: Bull. Soc. path. exot., 1909, ii, 288.
3. Brumpt: Nouveau Traité Médecine [Roger, Widal, and Teissier], 2. ed., Paris, 1922, iv, 491.

### Roumanian fowl spirochetosis

Described by Mezinescu and Colinescu. The animals have the usual symptoms—diarrhea, loss of appetite, paresis, fever, temperature 43.5° to 43.7° C. Incubation period four to six days. Blood full of spirochetes like the Brazilian parasites. In survivors from a first attack another attack develops eight to twelve days after. The disease disappears in winter, then appears after the first few days of hot weather. The virus is virulent in the blood in twenty-four hours, disappears in four to five days at time of crisis.

Transmitted to fowls, geese, ducks, and young turkeys. Mortality low in the experimental disease.

Mezinescu and Colinescu: Bull. Soc. path. exot., 1909, ii, 293.

### Spironema suis (King, Baeslock, and Hoffmann)

Described originally by King and Baeslock[1] from the blood of hogs with hog-cholera. Observations confirmed by King, Baeslock, and Hoffmann[2] who found the spirochetes in the blood of 40 animals suffering from the same disease. The organism was named by them *Spirochæta suis*. Subsequently found by King and Hoffmann[3] in the intestinal ulcer crypts in the cecum and in the external local lesions. Renamed *Spirochæta hyos* by King and Drake.[4]

Relatively large organisms averaging 5 to 7 $\mu$ in length and 1 $\mu$ in width. Body of organism flexible, round, and blunt at the ends. Actively motile, revolving about its longitudinal axis. Motility undulating. Spirals fixed. Polar flagellum demonstrated in one organism by the India ink method. Cultivated anaërobically by King and Hoffmann on various media, especially horse serum containing a bit of fresh rabbit kidney.

1. King and Baeslock: J. Infect. Dis., 1913, xii, 39.
2. King, Baeslock, and Hoffmann: J. Infect. Dis., 1913, xii, 206.
3. King and Hoffmann: J. Infect. Dis., 1913, xiii, 403.
4. King and Drake: J. Infect. Dis., 1915, xvi, 54.

### Spironema Theileri (Laveran)

Discovered by Theiler[1] in the blood of cattle from Pretoria, South Africa. First described by Laveran[2] in the blood of cattle sent to him by Theiler. Observations confirmed by Ziemann in Cameroon and by Koch in East Africa. Named *Spirochæta Theileri* by Laveran.[3]

Small spiral organisms, 7 to 10 $\mu$ long in the shorter forms, and 20 to 30 $\mu$ in the longer forms. Thickness $\frac{1}{4}$ to $\frac{1}{3}$ $\mu$. Organisms frequently bent or curved upon themselves, forming a complete circle or a loop or knot. Ends thinner and tapering. Number of spirals variable. Motility very active, organisms moving in all directions. Stains by thionin, methylene-blue, carbolfuchsin, anilin gentian-violet, by Laveran's stain for hematozoa (silver oxid, eosin, and tannin), and by Laveran's modification of Romanowsky Azur II. The last two stains do not reveal chromatic bodies like those found in protozoa.

Blood of cattle with spirochetosis infectious for oxen and sheep, not for goats and horses. Incubation period two to three days. Transmitted by the blue tick *Boöphilus decoloratus* (*Rhipocephalus decoloratus*) (Theiler[4]). Incubation period thirteen to seventeen days. Transmitted also by larvæ of *Rhipocephalus evertsi*. Laveran and Vallée[5] allowed blue ticks obtained from the Transvaal to bite a cow in Paris. The animal developed a double infection with spirochetes and *Piroplasma bigeminum*.

1. Theiler: J. Comp. Path. and Therap., 1904, xvii, 47.
2. Laveran: Compt. rend. Acad. d. sc., 1902, cxxxv, 717.
3. Laveran: Compt. rend. Acad. d. sc., 1903, cxxxvi, 939.
4. Theiler: Proc. Roy. Soc., London, Ser. B., vol. lxxvi, No. B512, p. 504.
   ———: Bull. Soc. path. exot., 1909, ii, 293.
5. Laveran and Vallée: Compt. rend. Acad. d. sc., 1905, cxl, 1515.

### SPIRONEMA BOVIS-CAFFRIS (Nuttall)

Described originally as *Spirochæta bovis-caffris* by Nuttall from the blood of a buffalo. Very broad and tapering at both ends. When stained by Giemsa, they are violet or violet-blue and contain very fine chromatin granules. Transverse achromatic bands seen in one specimen. These spirochetes measure 20 to 25 $\mu$ in length by 1 to 1.5 $\mu$ in width.

Nuttall: Parasitology, 1910, iii, 108.

### SPIRONEMA EQUI (Novy and Knapp)

Described originally by Theiler[1] in the blood of a horse in the Transvaal and named *Spirochæta equi* by Novy and Knapp.[2] Spirochetes were subsequently found in the horse by Martin[3] in Timbo, French Guinea, and by Stardy[4] in the British East African Protectorate.

The organisms are 12 to 15 $\mu$ long and about 0.25 $\mu$ thick (Martin). They generally have three to four open spirals with well-marked undulations. They roll up into rings or interrogation points. Forms with five to six spirals show fine pale, central areas indicating transverse division. Not transferred by Martin to sheep or hens. Spirochetes from horses transferred by Doddi[5] to sheep and oxen, the period of incubation being the same as that given by Theiler. Dodd believes that the spirochetes from sheep, horse, and oxen are all the same, *Spirochæta theileri.*

1. Theiler: J. Comp. Path. and Therap., 1904, xvii, 47.
2. Novy and Knapp: J. Infect. Dis., 1906, iii, 291.
3. Martin: Compt. rend. Soc. de biol., 1906, lx, 124.
4. Stardy: J. Comp. Path. and Therap., 1906, xix, 226.
5. Dodd: J. Comp. Path. and Therap., 1906, xix, 318.

### SPIRONEMA OVIS (Novy and Knapp)

Observed originally by Theiler[1] in the blood of sheep in the Transvaal and named *Spirochæta ovis* by Novy and Knapp.[2] Similar organisms described by Martoglio and Carpano[3] at Erythrea on the Red Sea. This organism was named *Spirochæta ovina* by Blanchard.[4] It was 10 to 20 $\mu$ long, 0.2 to 0.4 $\mu$ thick, and had 3 to 10 large regular turns. Ends thin or pointed. No flagella. Gram-negative. Not cultivated.

Dodd[5] believes that the spirochetes of sheep, horses, and oxen are all the same, *Spirochæta Theileri.* Theiler holds that the cattle and sheep spirochetes may be identical, but that the horse spirochetes are different.

1. Theiler: J. Comp. Path. and Therap., 1903, xvii, 55.
   ———: Proc. Roy. Soc., London, Ser. B., 1905, lxxvi, 504.
2. Novy and Knapp: J. Infect. Dis., 1906, iii, 291.
3. Martoglio and Carpano: Ann. d'ig. sper., 1904, xiv, 577.
4. Blanchard: Semaine Méd., 1906, xxvi, 1.
5. Dodd: J. Comp. Path. and Therap., 1906, xix, 216.

### SPIRONEMA MACACI (Castellani and Chalmers)

Described by Castellani and Chalmers in 1910. Originally found by them in monkeys in Ceylon in 1906. Measures about 12 $\mu$ in length. Closely resembles *Spironema Carteri.* Easily transferred from monkey to monkey.

The spirochetes found by Leishman, Balfour, and Plimmer in *Cercopithecus sebæ* from Sierra Leone may be a different species.

Castellani and Chalmers: Manual of Tropical Medicine. 3. ed. London, 1919, p. 453.

### SPIRONEMA PITHECI (Thiroux and Dufougeré)

Observed originally as a new spirillum in *Cercopithecus patas* by Thiroux and Dufougeré in the French Sudan and named by them *Spirillum pitheci.*

It resembles *Spironema Duttoni* of tick fever. Measures 15 to 20 $\mu$ in length by 0.25 $\mu$ in thickness and has five to six open spirals. Organisms occasionally rolled upon themselves, forming a complete circle. Causes an irregular febrile illness in *Cercopithecus patas* and in the febrile periods appears in the circulating blood. Easily transmitted to mice, in which it appears two to six days after inoculation. Death follows in twenty-five to twenty-six days. Less virulent to rats. Not virulent to cold-blooded animals (serpents).

Thiroux and Dufougeré: Compt. rend. Acad. d. sc., 1910, cl, 132.

### SPIRONEMA LUTRÆ (Prowazek)

Found originally by Prowazek in the blood of the otter (*Lutra starr*) and described as *Spirochæta lutræ.* Wide ribbon-formed spirochete with blunt ends. Protoplasm stains blue by Romanowsky-Giemsa and shows often four large isolated chromatin fragments. In rare instances the nucleus gives off chromatic granules.

Prowazek: Arb. a. d. K. Gsndhtsamte, 1907, xxvi, 23.

### SPIRONEMA CAVIÆ (Macfie)

Found in the blood of a guinea-pig at Lagos by Macfie and described as a spirochete.

Length 2 to 9 $\mu$. Crest of curve occurs at 5 $\mu$. Double forms, two individuals, 14 $\mu$ in length. Undulations vary from 2 to 9, majority have 3, 4, or 5 undulations. Cultivated in a mixture of 2 c.c. of the infected guinea-pig's blood poured into 1/10 c.c. of 50 per cent. aqueous solution of glucose.

Macfie: Ann. Trop. M. and Parasitol., 1914, viii, 439.

### SPIRONEMA VESPERTILIONIS (Novy and Knapp)

Found first by Nicolle and Comte[1] in the blood of the Tunisian bat, *Vespertilio Kuhlii,* and named *Spirochæta vespertilionis* by Novy and Knapp.[2]

Length 12 to 18 $\mu$. Thickness 0.25 $\mu$. Ends very thin or pointed. Stains by carbol thionin, Laveran's, Leishman's, and Giemsa's methods. Division transverse. No undulating membrane. Protoplasm homogeneous. Actively motile. Movements progressive and vibratory with a contraction of the body. The blood is infectious to normal bats by intraperitoneal inoculation. The animals show an incubation period, an acute fever, and usually recover. After an experimental attack an immunity develops to subsequent inoculation. Blood not infectious to white mice or monkeys.

1. Nicolle and Comte: Compt. rend. Soc. de biol., 1905, lix, 200.
   ———: Ann. de l'Inst. Pasteur, 1906, xx, 311.
2. Novy and Knapp: J. Infect. Dis., 1906, iii, 291.

### SPIRONEMA VESPERUGINIS (Gonder)

First seen by Gonder[1] in the blood of *Vesperugo Kuhlii,* the Tunisian bat. Different from the organisms of Nicolle and Comte.[2] Gonder's spirochetes varied in length from small forms showing 1 to 3 spirals, 3 to 5 $\mu$ long, to large forms measuring 30 $\mu$. The usual individuals measured 10 to 20 $\mu$. The longest individuals may have been two spirochetes adhering after longitudinal division. Spirals very irregular and lacking in uniformity.

**Motility** rapid bending, corkscrew, sinuous, and rotating, both backward and forward. Resting forms bent, stretched out, or slightly wound in spirals. The ends terminate in a

point, a kind of flagellum, which is a prolongation of the periplast. This periplast is stretched in the form of an undulating membrane over the entire cell. It is best observed when the organisms are resting or when they are killed in glycerin. Prowazek's observation of an independent movement of the membrane is fully verified by Gonder. No plasmolysis in 2 to 5 per cent. NaCl. Killed rapidly and dissolved by caustic potash and by glycerin.

The organisms often contain refractile bodies easily stained by a double Giemsa. The first staining is carried out in a mixture of eosin (2.5 c.c. of a 1 per cent. solution in 500 c.c. water) with azur II (1 : 1000) (40 parts of eosin and 8 to 10 parts azur), the second with a mixture of eosin (as above) and azur I (0.8: 1000) (40 parts of eosin and 5 to 8 parts of azur). These highly refractile bodies are probably chromatin. A nuclear configuration is also found in these spirochetes like that described for *Balbianii* by Perrin, for *anodontæ* by Keyselitz, and for *gallinarum* and *dentium* by Prowazek.

The cell body is often stained dark red in good preparations, lighter at the ends or on one side. Chromatin substance may possibly be arranged as a fine chromidial network. Often the chromidia appear as very fine granules, irregular fragments, and short rods arranged in pairs. Sometimes larger deeply stained, dark red, nuclei appear or several darkly stained granules. These correspond to the highly refractile bodies in the living organisms. The chromatic substance also appears as a long rod staining dark red with Giemsa, often with minute particles next it. Rarely organisms with protoplasmic buttons may be seen. The exact significance of these nuclear bodies or the nuclear configuration is not clear since, it is not possible to follow the nuclear changes in the living cells. Nevertheless one can find nuclei and chromatin rods like those known for other spirochetes.

An undulating membrane was demonstrated also by the double Giemsa, appearing bright red with a short flagellum at either end.

Increase by longitudinal division beginning at one end and extending through the organism. Spirochetes often found attached to each other by the ends. In dividing equal quantities of chromatin granules go to each daughter-cell.

Spirochetes rarely found in the blood-corpuscles.

Possibly transmitted by species of tick belonging to ixodiden, with which the bats are infected. Not proved experimentally.

Gonder's observations on morphology and longitudinal division have not been confirmed.

1. Gonder: Arb. a. d. K. Gsndhtsamte, 1908, xxvii, 406.
2. Nicolle and Comte: Compt. rend. Soc. de biol., 1905, lix, 200.

### Spironema tropidonoti (Dobell)

Found once by Dobell in the blood of a snake, *Tropidonotus stolatus*. They were rare and difficult to find. The living organisms appeared slender, flexible, and corkscrew-like, actively motile, closely resembling *Spirochæta Duttoni* in general form. They measured about 15 $\mu$ in length and about 0.5 $\mu$ in breadth and stained uniformly pink by Giemsa. They probably divided transversely.

Dobell: Spolia Zeylanica, 1910, vii, 65.

### Spironema minor (Carter)

Described in the blood of an Indian rat, *Mus decumanus*, by Carter,[1] and found once in 117 animals.

Minute organism like *Spirochæta Obermeieri*. In dried and anilin-stained preparations it appears as an extended and uniformly slender filament of clearly spiral construction, having a length commonly less than the diameter of a red blood-corpuscle, varying from 5 to 9 $\mu$ (1/5000 to 1/3000 inch). Varying with the length four to eight close spiral turns appear. Exceedingly slender, both ends seem a little pointed. No flagellum detected. Homogeneous structure. Best preparations obtained by rendering blood translucent by strong acetic acid or solution of potash before staining. Motility active and tolerably sustained, rotation about long axis, propulsion backward or forward, occasionally an energetic lateral twisting or lashing. During movement slight unwinding of the screw seems to take place, with consequent elongation. Rotation may occur without change of position. In hermetically sealed drops of blood, pure or diluted with $\frac{1}{2}$ per cent. NaCl solution, the movements become languid and more rotatory. At the end of twenty-four hours the organisms are quiescent, with filaments dotted. After forty to fifty hours the organisms cease to be visible. No growth in blood outside body.

Not definitely associated with pyrexia in the rat except that the spirochetes declined with

subsidence of fever and returned to the blood when the fever came back. Animal regarded as "septic" by Carter.

Not transmitted to rats or monkeys, *Macacus rhesus* (Wenyon[2]).

1. Carter: Scientific Memoirs by Medical Officers of the Army of India. Part III, 1887, p. 45.
2. Wenyon: J. Hyg., 1906, vi, 580.

### SPIRONEMA LAVERANI (Breinl and Kinghorn)

Noted originally as *Spirochæta Laverani* by Breinl and Kinghorn[1] from the blood of a white mouse.

Very small translucent actively motile spiral organism showing both rotatory and progressive movements. Stains uniformly and readily with the anilin dyes, and the various modifications of the Romanowsky stain.

Measures 0.1 to 0.2 $\mu$ in breadth and 1.8 to 3.75 $\mu$ in length. Number of spirals two to four. Pointed at both ends.

Not transmitted to other mice.

A similar spiral organism found in the blood of a wild mouse, *Mus musculus*.

The spiral organism described by Vandyke Carter[2] in the blood of an Indian rat is longer and possesses more spiral turns.

1. Breinl and Kinghorn: Lancet, 1906, ii, 651.
2. Carter: Scientific Memoirs by Medical Officers of the Army of India. Part III, 1887, p. 45.

### SPIRONEMA MURIS (Wenyon)

Found originally and named *Spirochæta muris* by Wenyon[1] in the blood of a brown mouse.

Very active spiral organism turning on its long axis and showing darting motility from either end. It also spins rapidly about its central point. Spirals permanent and the body fairly rigid. Motility suggests flagella which, however, were not seen. No evidence of an undulatory membrane. Stains uniformly with a clear unstained central spot. Ends slightly tapering. No nucleus or undulatory membrane in stained preparations. Division apparently transverse. No evidence of longitudinal division. Number of turns two to six. Width about 0.2 $\mu$. Length varies from 3 to 4 to 6 to 7 $\mu$.

Transmitted from mouse to mouse. The organisms appear in the peripheral blood five to six days after infection and remain there for some weeks, without doing any apparent injury to the animal. Also transmitted to young rats. Similar spiral organisms found in lice which infested the mice, but attempts to produce infection in mice by means of the lice were not successful. Not cultivated.

MacNeal[2] states that the spiral organism found by Borrel[3] in mouse carcinoma has been shown by Wenyon to be identical with his *Spirochæta muris*.

1. Wenyon: J. Hyg., 1906, vi, 580.
2. MacNeal: Proc. Soc. Exper. Biol. and Med., 1907, vi, 125.
3. Borrel: Compt. rend. Soc. de biol., 1905, lviii, 770.

### SPIRONEMA MURIS var. VIRGINIANA MacNeal

Noted by MacNeal[1] as *Spirochæta muris* var. *Virginiana* and regarded by him as probably a variety of *Spirochæta muris* Wenyon. Found in the blood of a wild rat caught at Morgantown, West Virginia.

Minute, actively motile, spiral organism rotating on its long axis and darting forward and backward. It also shows flexuous movements of the whole body. Stains readily by the various modifications of the Romanowsky stain and intensely by MacNeal's rapid method for staining *pallidum*.[2] Takes a uniform deep violet-red color. Varies in length from 1.75 $\mu$ with one and three-quarter turns or nodes to 3.55 $\mu$ with three and a half turns. Spirals 3.75 to 4 $\mu$ long are seen with central constrictions suggesting transverse division. Thickness 0.25 $\mu$, the gross width of the coil about 0.65 $\mu$. Length of a turn or node, crest to crest, 1 to 1.5 $\mu$. The spirals correspond to an ordinary right-hand screw turning clockwise from a given point.

Infection easily transmitted to other wild rats which harbor the parasites for periods

varying from one to nine days, recover, and then develop repeated relapses. The animals do not die. White rats are also susceptible and rarely house mice, *Mus musculus*.

1. MacNeal: Proc. Soc. Exper. Biol. and Med., 1907, iv, 125.
2. MacNeal: J. Am. M. Ass., 1907, xlviii, 609.

### Spironema Jonesii (Dutton, Todd, and Tobey)

Described originally as *Spirochæta Jonesii* by Dutton, Todd, and Tobey from the blood of fresh water fish, *Clarias angolensis*.

Length 18 μ. Breadth at widest part 0.6 μ. Widest at one extremity or toward the middle, in which case the organism tapers toward both ends, but one extremity is always thicker than the other. The wide end is rounded and the other drawn out to a fine and slender point. Usually occurs singly and appears as a simple curve, three-quarters the circumference of a circle. Also appears in compound curves and spirals. Coils always formed by the small end of the organism. Spiral forms long, 24 to 27 μ, with an occasional suggestion of a membrane. Also in pairs and groups. The organisms seem to consist of a deeply staining zone and a lightly staining periplast. Stained irregularly by modifications of the Romanowsky stain. Granular forms occurred with irregularly distributed granular masses in the protoplasm.

Dutton, Todd, and Tobey: Ann. Trop. Med. and Parasitol., 1907, i, 287.
———: J. Med. Research, 1906, xv, 491.

### Spironema latapici (Laveran)

Found by Laveran in the blood of a shark in Australia. The organism shows 3 to 4 spiral turns with well-marked spirals or may be almost straight. When straight it is 40 μ long. Thickness about 1 μ. One end pointed, one end round. Some forms doubled and curved or twisted about themselves. No details of structure made out by staining. Dividing forms not seen.

Laveran: Bull. Soc. path. exot., 1908, i, 149.

### Spironema gadi (Neumann)

Described originally as *Spirochæta gadi* by Neumann from the peripheral blood and internal organs of the sea fish, *Gadus minutus*.

Very irregular in length, thickness, and the width of its curves which may be few or many. Average length 10 to 16 μ, extremes 5 and 22 μ. Openings of curves or spirals 3.5 to 4 μ wide. Height of curve or spiral about 1 μ. Openings may be 2 or 6 μ. Thickness 0.1 to 0.2 μ. Resembles *Spironema gallinarum* and *Spironema Duttoni*. Somewhat thinner than the latter. Motility active from place to place with turning about the axis of the organisms. Also forward and backward movements. Stains with fuchsin, methylene-blue, gentian-violet and Giemsa. No definite periplastic processes, no separation into protoplasm and chromatin. By Giemsa the organisms appear sharply pointed on both ends.

Neumann: Ztschr. f. Hyg., 1909, lxiv, 1.

### Spironema pelamidis (Neumann)

Observed by Neumann in the blood of the sea fish, *Pelamys sarda*, and named *Spirochæta pelamidis*.

Shorter and more delicate than *Spironema gadi*. Usual length 9 to 10 μ, but forms 6 μ long and forms 15 to 16 μ long are also present. Majority 0.25 μ thick, some only 0.1 μ thick. Long organisms appear as if about to divide transversely. Ends thin and pointed. The organisms usually have fine spiral curves, regular in length and height. Height of the curves about 0.5 to 0.8 μ. Breadth of curves 1 to 1.5 μ. Both height and width of curves less than those of *Spirochæta gadi*. Motility fairly active, backward and forward. In ten to twenty seconds the organisms move across a field of the microscope at a magnification of 1000. Movements boring and turning, the curves of the organism remaining the same. Also a turning about the axis of the organisms occurs and an undulation of the curves without change of location. Stains by fuchsin, methylene-blue, gentian-violet, and Giemsa.

Neumann: Ztschr. f. Hyg., 1909, lxiv, 1.

### SPIRONEMA POLLACHII (Henry)

Noted by Henry[1] as *Spirochæta pollachii* from the blood of the pollack, *Gadus pollachius*.

Short forms from 8 to 13 $\mu$ long, averaging 11.5 $\mu$, from about 50 per cent. of the specimens. They are usually quite straight, but may be curved or sickle-shaped. They show less deeply staining pointed extremities. Average number of spirals about 7.7 and each spiral about 1.5 $\mu$ long. Long forms vary in length from 15 to 20 $\mu$, average length between 17 and 18 $\mu$. In these long forms the spirals average 10.4 in number, each being about 1.6 $\mu$ in length. The longer forms thus show an increase in number of spirals. The long forms make up about 38 per cent. of the total. Transverse division indicated by thinned-out central portions.

Corpuscular inclusions appear in the blood which may represent encysted stages of the spirochetes. They are small circular bodies, 0.5 to 2 $\mu$ in diameter, lying between the nucleus and the periphery of the corpuscular protoplasm. They take on a solid purple staining like the chromatin of the nucleus. Sometimes two such bodies appear, and sometimes these are joined by a strand presenting a dumb-bell appearance. Swollen spherical forms also occur, staining diffusely blue. These corpuscular bodies resemble the so-called intracorpuscular stages of the fowl spirochete described by Balfour[2] in the spirochetosis of Sudanese fowls.

1. Henry: J. Path. and Bacteriol., 1912–13, xvii, 160.
2. Balfour: Third Rep. Wellcome Tropical Research Lab., Khartoum, 1908.

## 5. GENUS TREPONEMA Schaudinn

Type species *Treponema pallidum*, Schaudinn and Hoffmann.

Pathogenic and non-pathogenic varieties. Tissue parasites. Length 6 to 14 $\mu$, pointed ends. Diameter 0.25 to 0.3 $\mu$. Body cylindrical. Spirals regular rigid. Spiral amplitude 1 $\mu$. Spiral depth 0.8 to 1 $\mu$. One or more slightly undulating wavy curves may be present. Whole organism seems to consist of a spirally wound axial filament. Membrane doubtfully present. If it exists, it must be flexible. Fine terminal spiral filament, easily seen in cultures. No chambered structure, crista, flagella, or highly motile end-portion. Division transverse. Membrane cannot be separately stained. Body stains pink by Giemsa. Resists tryptic digestion many days. Complete disintegration in bile salts (10 per cent.). Slow disintegration in saponin (10 per cent.).

The treponemata may be three-dimension spirals as distinguished from two-dimension spirals like spironemata.

*Man*

Type species *Treponema pallidum* (Schaudinn and Hoffmann).
" *rigidum* (Zinsser and Hopkins).
" *refringens* (Schaudinn and Hoffmann).
" *pertenue* (Castellani).
" *obtusum* (Castellani).
" *acuminatum* (Castellani).
" *aboriginale* (Cleland).
" *buccale* (Lühe) Dobell.
" *dentium* (Lühe) Dobell.
" *intermedium* Dobell.
" *microdentium* Noguchi.
" *macrodentium* Noguchi.
" *gracile* (Vesprémi).
" *mucosum* Noguchi.
" *calligyrum* Noguchi.
" *gracile* (Levaditi and Stanesco).

Type species *Treponema minutum* Noguchi.
" *phagydenis* Noguchi.
" *balanitidis* (Hoffmann and Prowazek).
" *Vincenti* (Blanchard).
" *bronchiale* (Castellani).
" of Nolf and Spehl.
" *Schaudinni* (Prowazek).
" *eurygyratum* (Werner).
" *stenogyratum* (Werner).

*Rabbits*

*Treponema cuniculi* (Jacobstal) Noguchi.

*Toads*

*Treponema bufonis* (Dobell).
" *minutum* (Dobell).

*Cockroaches*

*Treponema stylopygæ* Dobell.
" *parvum* Dobell.

*Termites*

*Treponema termitis* (Leidy).
" *minei* (Prowazek) Dobell.
" *Grassi* Doflein.

*Flies*

*Treponema glossinæ* Novy and Knapp.

*Fleas*

*Treponema ctenocephali* (Patton).

*Mosquitoes*

*Treponema culicis* (Jaffé).

*Mussels*

*Treponema Hartmanni* (Gonder).

*Water*

*Treponema vivax* Dobell.

### TREPONEMA PALLIDUM (Schaudinn and Hoffmann)

Discovered by Schaudinn and Hoffmann[1] in 1906 in primary and secondary lues and described by them as *Spirochæta pallida*. Subsequently they changed the name to *Treponema pallidum* as it is usually known.

**Morphology.**—Delicate undulating filaments from 4 to 10 $\mu$ in length and up to 0.5 $\mu$ in thickness. Usually about 7 $\mu$ long. Smaller and more delicate than the spirochetes in relapsing fever. Schaudinn and Hoffmann state that the organism consists of three to twelve convolutions joined at acute angles. Ends flattened or pointed. Round on cross-section (Besson). Coiled forms of *pallidum* have been described by Prowazek[2] and Meirowsky,[3] who regard them as resting bodies or resisting forms. In films of *pallidum* from lesions granules have been demonstrated by Fantham, who believes that their presence may explain parasyphilis. Such granules have been demonstrated by Noguchi[4] in pure cultures. Dissolved in sodium taurocholate 1 : 10 (Prowazek).

Fig. 179.—*Treponema pallidum*, Nichol's strain, from thirteen-day ascitic agar culture, dark field illumination.

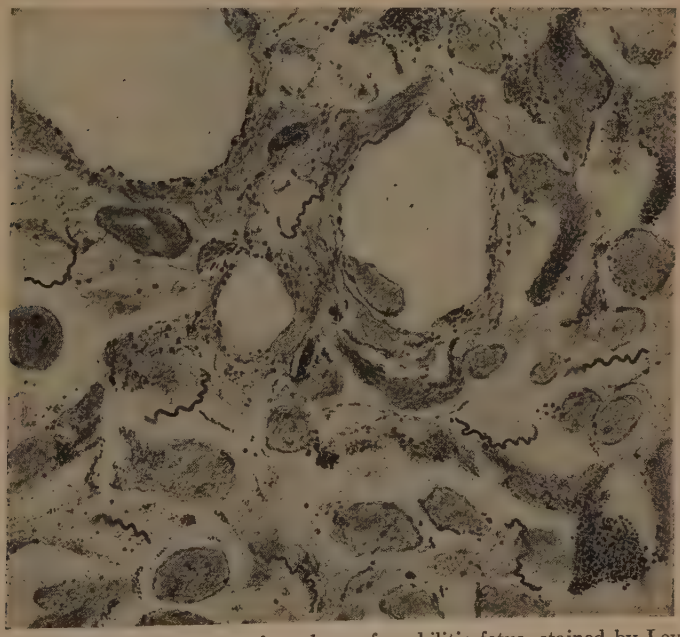

Fig. 180.—*Treponema pallidum* from lung of syphilitic fetus, stained by Levaditi.

**Cultivation.**—The cultivated *pallidum* is short and actively motile in young cultures, after two weeks having its usual length with eight to twelve curves. The mature organisms are still active with a corkscrew motility. The ends show fine flagella-like prolongations which are frequently perfectly curved or may be devoid of curves (Noguchi[5]). Noguchi[6] has differentiated three different types of morphology in culture *pallidum*, the thick type, the thin type, and the average type. The thin type resembles *Treponema microdentium*, especially when it has been under cultivation two to three weeks, but requires tissue in the medium and does not produce a putrefactive odor. The average type may be regarded as the normal. Inoculation with it produces a diffuse induration and swelling in the testicles of rabbits, usually within three weeks. This progresses steadily for five to six weeks, then undergoes spontaneous retrogression. The thick type produces small localized hard nodules five to six weeks after inoculation, reaching their maximum size in eight to ten weeks. These nodules are cartilaginous in consistence and contain a greater amount of mucin. The thinner type produces a diffuse testicular swelling within ten to fourteen days, progressing rapidly for five to six weeks. The observations of Noguchi were not confirmed by Zinsser, Hopkins, and McBurney,[7] who were not able to correlate pathogenicity or invasive property with

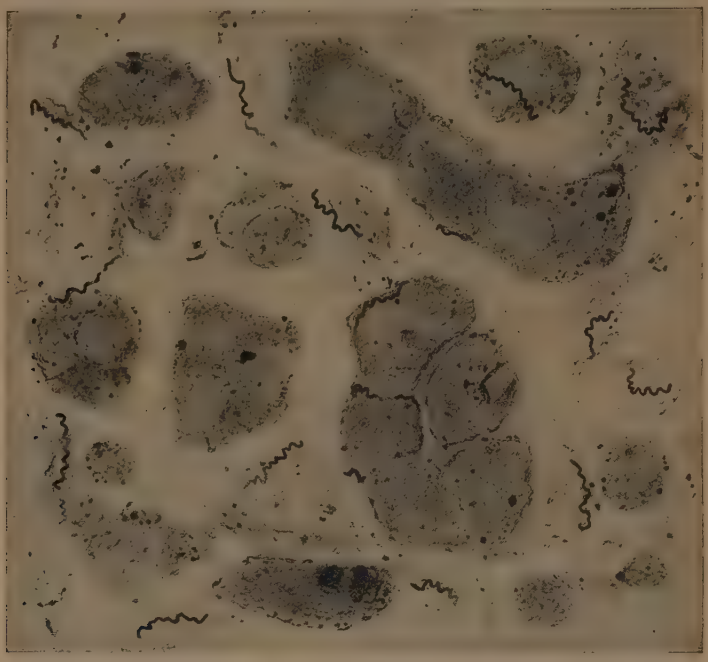

Fig. 181.—*Treponema pallidum* from liver of syphilitic fetus, stained by Levaditi.

differences in size. According to these authors the nature of the lesions in experimental syphilis in rabbits from cultivated *pallidum* depends upon fortuitous circumstances, age of rabbits, etc.

**Motility** active, backward and forward, rotating about the long axis and occasionally bending.

**Division** transverse. Prowazek's early observations of longitudinal division have now been disproved.

**Methods of Demonstration.**—Unstained *Treponema pallidum* may be seen best by dark field illumination with the ordinary microscope. It stains badly by ordinary methods, such as carbolfuchsin and gentian-violet. It stains well by Giemsa's azur-eosin by the method recommended by Schaudinn and Hoffmann. It may also be stained by a variety of other methods, those of Fontana, Levaditi and Monuelian, and Wood giving excellent results. It may be demonstrated easily by Burri's India ink method.

**Cultivation.**—Early attempts at cultivation of *Treponema pallidum* by ordinary bacteriological methods uniformly resulted in failure. Schereschewsky,[8] by the use of sterile horse serum coagulated at 60° C. and bits of syphilitic tissue, may have had some multiplication of the organism in his cultures. Mühlens[9] and Hoffmann,[10] who used media without

tissue, probably brought to cultivation other spirochetes and not the virus of syphilis. The first successful cultivation of genuine *pallidum* was accomplished by Noguchi,[5] who employed high cylindrical layers of solid media (about 10 c.c.) consisting of 2 parts of 2 per cent. slightly alkaline agar and 1 part of ascitic or hydrocele fluid containing a fragment of sterile tissue, rabbit's kidney, or testicle. The medium was covered by a layer of paraffin oil 3 cm. deep, and incubated under strict anaërobic conditions. The inoculations were made from lesions rich in *pallidum*, such as chancres, condylomata, skin papules, or the testicles of rabbits infected experimentally with lues. The organisms developed as a faint, hazy zone radiating from the stab toward the sides of the tube. The contaminating bacteria grow along the canal of the stab. Pure cultures were obtained by transferring the organisms from the hazy zone to fresh tubes. Colonies of *pallidum* are not usually sharply defined, but are always faintly visible. Discrete colonies are rare, but may occur when the ascitic fluid is favorable. Growth is most marked in the immediate vicinity of the fragments of sterile tissue. Horse serum or sheep serum may be used, but they are not so good as ascitic or hydrocele fluid. The growth appears in about seventy-two hours and increases for two to three weeks, when it is at its height. The tissue apparently acts by removing the traces of oxygen in the lower levels of the medium and also by furnishing some special form of nutriment. Cultivation was likewise accomplished in liquid medium by Noguchi[11] who used tubes containing 10 c.c. of serum water made of 3 parts of distilled water and 1 part of horse sheep, or rabbit serum, each tube containing a small piece of sterile rabbit kidney or testicle or a bit of syphilitic testicle. The tubes were covered with paraffin oil and incubated anaërobically. Here the *pallidum* grew mixed with other organisms, pure cultures being obtained by allowing the spirochetes to grow through Berkefeld filters. The growth in liquid media was very slow and continued indefinitely for many weeks. The medium remained fluid and developed no putrefactive odor. The organisms cultivated by Noguchi were not the same as those cultivated by Mühlens and Hoffmann. They were pathogenic for *Macacus rhesus* and *Cercopithecus callitrichus*. Inoculations of ten-day cultures of ascitic fluid tissue agar to freshly shaven scarified surface of the skin over the eyebrows, and also upon the scarified surface of the prepuce, scrotum, and labia, produced primary lesions like those found in man and produced in monkeys by serum containing spirochetes from human sources. The blood of the inoculated monkeys developed positive Wassermann reactions.

After the *pallidum* has been obtained pure by the Noguchi method it may be kept alive best in a mixture of sterile hydrocele or ascitic fluid and broth containing tissue.[12] This is placed in one tube connected to another in which the solid medium containing tissue is placed. Both the solid and fluid media are inoculated with the pure culture. The organisms grow in the solid medium and then appear in the fluid medium when proper conditions for its growth are established. The virus of syphilis has subsequently been cultivated by numerous observers who have employed the method of Noguchi or some modification of it. Thus Zinsser and Hopkins[13] cultivated it in ascitic broth containing heated or unheated rabbit kidney incubated anaërobically, and in ascitic broth containing a bit of slanted egg medium with or without glycerin, under paraffin oil. Zinsser, Hopkins, and Gilbert[14] have grown *pallidum* in fluid media without the addition of agar when tissues sterilized by heat are added, the best growth being obtained with lung and suprarenal gland. The mixtures consist of slightly acid broth of an acidity of 0.2 to 0.8 per cent., sheep, horse, or rabbit serum, or ascitic fluid, and autoclaved tissue, rabbit kidney, liver, spleen, brain, lung, heart, and skeletal muscles. Akatsu[15] has shown that carbohydrates added to the media have no especial value in enhancing the growth.

*Treponema pallidum* has likewise been grown by Shimanine in his special medium containing sodium nucleate, and by Weiss and Wilkes-Weiss,[16] who have employed a kind of "hormone" broth to which egg is added.

The optimum reaction of media for the cultivation of the spirochetes of lues is *p*H 7.2 to 7.6 (Kranz[17]).

**Pathogenic Action.**—Metchnikoff and Roux[18] in 1903 succeeded in transmitting syphilis to monkeys, producing primary lesions on the clitoris of the female chimpanzee. Twenty-six days after inoculation a chancre developed with coincident swelling of the inguinal glands. In fifty-six days a secondary eruption appeared with a swelling of the spleen and lymph-nodes. Successful results were also obtained by Lasser and by Nicolle.[19] Small bits of syphilitic tissue from primary and secondary lesions were employed, not material from tertiary lesions. With syphilitic material Bertarelli and Volpino[20] produced lesions in the cornea of rabbits and Parodi lesions in the testicles of rabbits, this being confirmed by Uhlenhuth and Mulzer.[21] With his original cultures Noguchi produced typical syphilitic lesions in the testicles of rabbits, the organisms being demonstrated in the tissues. Brown and Pearce[22] finally have produced lesions of almost all types by various methods of inoculation, using syphilitic animal material.

Zinsser, Hopkins, and McBurney[23] have found that cultivated *pallidum* does not suffer any change in pathogenic action during rabbit passage. No difference in pathogenicity could be found in the strains isolated from condylomata, chancres, mucous patches, and by Nichols from the nervous system. The different strains showed no difference in speed of

incubation. Cultivated *pallidum* loses its pathogenic action rapidly and virulent cultures do not induce antibody formation in rabbits. Moreover, cultivated pallidum and syphilitic testes do not act as antigens in the Wassermann reaction.

*Antibodies* to cultivated *Treponema pallidum*. It has been shown by Kolmer[24] that agglutinins can be produced for cultivated *pallidum* by the intravenous administration of young cultures of living spirochetes. These have a strength of 1:1280, normal rabbit serum not agglutinating in a dilution as low as 1:20. Agglutinins were also produced in rabbits by the inoculation of living material into the testicles and were active in a dilution of 1:320. Complement-fixing bodies were demonstrable in rabbits immunized by both these methods, salt solution and alcoholic extracts of the cultures being used as antigen. Kolmer also showed that there was no appreciable amount of agglutinin for culture *pallidum* in the sera of secondary or tertiary syphilis or in the cerebrospinal fluid of tertiary syphilis in dilutions of 1:20 up to 1:640.

It was subsequently pointed out by Zinsser and Hopkins[25] that agglutinins for *pallidum* may be produced in rabbits and sheep by systemic inoculation of concentrated culture material. The serum acquires spirocheticidal (treponemacidal) properties for cultivated spirochetes and some similar property exists in normal serum. Both the normal and immune treponemacidal serum is inactivated at 56° C. and may be reactivated by the addition of fresh normal serum from the same species. The treponemas thus act like bacteria. The agglutinins may be as strong as 1:4000. These agglutinating and treponemacidal substances against cultural *pallidum* have no action, at least in rabbits and sheep, upon virulent treponemata obtained directly from lesions. According to Noguchi and Akatsu[26] the agglutinins for *pallidum* disappear rapidly from the blood after cessation of immunization. Complement-fixing principles and treponemacidal substances were also observed in the sera of immunized animals. The immune bodies for culture *pallidum* had no effect upon virulent *pallidum*.

Finally it has been shown by Zinsser, Hopkins, and McBurney[27] that there is a close immunological relationship between cultivated *pallidum* and non-pathogenic treponemata like *Treponema calligyrum*. The sera of syphilitics agglutinates *pallidum* to a slightly greater degree than the serum of normal individuals. This has also been pointed out by Kolmer, Broadwell, and Matsunami,[28] who found some increase in the sera of patients with secondary and tertiary lues. Animals immunized with culture pallidum are apparently not immune to virulent pallidum.

**Occurrence.**—*Treponema pallidum* occurs regularly in the primary and secondary lesions of syphilis, usually in association with other spirochetes. The organisms have been found occasionally in the blood (Bandi and Simonelli,[29] Levaditi and Petresco[30]). They occur in the tertiary lesions as shown especially by Tomaszewski,[31] Ewing,[32] Noguchi and Moore,[33] and Warthin.[34] They are constantly present in the tissues of congenital syphilis, especially in the internal organs, lungs, liver, spleen, pancreas, kidneys, and occasionally in the heart muscle.

It is possible that there are several types of *Treponema pallidum* which are endowed with different abilities to invade the tissues. Thus Nichols[35] has isolated a special strain from the nervous system. Levaditi and Marie[36] believe that parasyphilis, tabes and general paralysis, is caused by a treponema distinct from that producing cutaneous and mucous membrane lesions. They call the former a neurotropic virus to distinguish it from the usual dermotropic virus. Fournier and Schwartz[37] have pointed out differences among the spirochetes obtained from chancres by the inoculation of rabbits.

**The Wassermann Reaction.**—This reaction, now almost universally employed for the diagnosis of syphilis, was originally proposed by Wassermann, Neisser, and Bruck[38] who employed as antigen extracts of syphilitic organs, preferably fetal syphilitic livers. It was supposed at that time that the free *Treponema pallidum* in these organs played the rôle of the active antigen which combined with the antibody in the patient's serum. The reaction was carried out with standardized complement and a properly prepared hemolytic system. It has now been shown that the substance which acts as the antigen is not the spirochetes in the fetal organs, but some lipoid material which may also be obtained from normal organs of man and animals, such as the beef heart. The reaction can no longer be regarded as a specific immune reaction. It has furthermore been shown by Zinsser, Noguchi,[39] Craig and Nichols,[40] and Kolmer that cultivated *Treponema pallidum* do not ordinarily act as antigen with the serum of syphilitic patients or syphilitic animals. The Wassermann reaction nevertheless is practically always given by the serum of undoubted luetic patients, and given so rarely by the serum of non-luetics that it has become a routine clinical diagnostic method of the greatest value.

Zinsser[41] regards as the best antigens for the Wassermann reaction beef, human, or guinea-pig heart, Noguchi's acetone-insoluble lipoid antigen, and chloresterinized antigen. The heart muscle is finely chopped and extracted in five times its volume of absolute alcohol. Noguchi's antigen is made from fresh spleen which is extracted in five times its volume of absolute alcohol for five to seven days at 37° C. This material is filtered, evaporated to dryness, and taken up in a small quantity of ether. The ether solution is then poured into

four times its volume of C. P. acetone. A flocculent precipitate forms which is now collected on a filter and preserved under acetone. Of this material 0.2 gram is dissolved in 5 c.c. of ether and shaken up with 100 c.c. of salt solution till the ether is evaporated. The resulting fluid contains active antigen which should be titrated with the serum of known syphilitic patients before use. The cholesternized antigen is made from human or guinea-pig heart to which 0.4 per cent. cholesterin is added (Walker and Swift[42]).

The hemolytic system usually employed is serum from rabbits treated with washed sheep corpuscles. This serum must be inactivated by heating to 57° C. half an hour and accurately titrated before use. The complement is practically always fresh guinea-pig serum. Blood is drawn under aseptic precautions from the animals, allowed to clot, and then centrifugalized to obtain a clear product. This serum is now examined for the presence of complement by testing it with mixtures of sheep corpuscles and hemolytic serum.

Several modifications of the original hemolytic system of the Wassermann reaction have been recommended, but none of them have met with the same success. The best of these is Noguchi's. Noguchi uses antihuman hemolytic serum and human erythrocytes for his hemolytic system. These erythrocytes may be obtained from the blood of the patient.


1. Schaudinn and Hoffmann: Arb. a. d. K. Gsndhtsamte, 1905, xxii, 527.
2. Prowazek: Arb. a. d. K. Gsndhtsamte, 1907, xxvi, 23.
3. Meirowsky: München. med. Wchnschr., 1913, vi, 1870.
4. Noguchi: Proc. Roy. Soc. Med., 1913, vii, 3.
5. Noguchi: J. Exper. M., 1912, xv, 90.
6. Noguchi: J. Exper. M., 1912, xv, 201.
7. Zinsser, Hopkins, and McBurney: J. Exper. M., 1916, xxiii, 329.
8. Schereschewsky: Deutsche med. Wchnschr., 1909, xxxv, 835, 1260.
9. Mühlens: Klin. Jahrb., 1910, xxiii, 339.
10. Hoffmann: Ztschr. f. Hyg., 1911, lviii, 27.
11. Noguchi: J. Exper. M., 1911, xiv, 99.
12. Noguchi: J. Exper. M., 1912, xvi, 211.
13. Zinsser and Hopkins: J. Exper. M., 1916, xxiii, 323.
14. Zinsser, Hopkins, and Gilbert: J. Exper. Med., 1915, xxi, 213.
15. Akatsu: J. Exper. M., 1917, xxv, 375.
16. Weiss and Wilkes-Weiss: J. Infect. Dis., 1924, xxiv, 212.
17. Kranz: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1924, xcii, Orig., 216.
18. Metchnikoff and Roux: Ann. de l'Inst. Pasteur, 1903, xvii, 809, etc.
19. Nicolle: Ann. de l'Inst. Pasteur, 1903, xvi, 631.
20. Bertarelli and Volpino: Centralbl. f. Bakteriol. [etc.], 1906, xl, 56.
21. Uhlenhuth and Mulzer: Arb. a. d. K. Gsndhtsamte, 1910, xxxiii, 183.
22. Brown and Pearce: J. Exper. M., 1920, xxxi, 475, etc.
23. Zinsser, Hopkins, and McBurney: J. Exper. M., 1916, xxiii, 329.
24. Kolmer: J. Exper. M., 1913, xviii, 18.
25. Zinsser and Hopkins: J. Exper. M., 1915, xxi, 576. *Ibid.*, 1916, xxiii, 323.
26. Noguchi and Akatsu: J. Exper. M., 1917, xxv, 765.
27. Zinsser, Hopkins, and McBurney: J. Exper. M., 1916, xxiv, 561.
28. Kolmer, Broadwell, and Matsunami: J. Exper. M., 1916, xxiv, 333.
29. Bandi and Simonelli: Centralbl. f. Bakteriol. [etc.], 1906, xl, 64.
30. Levaditi and Petresco: Presse Med., 1905, xiii, 617.
31. Tomasczewski: München. med. Wchnschr., 1906, liii, 1301.
32. Ewing: Proc. N. York Path. Soc., 1905–06, v, 105.
33. Noguchi and Moore: J. Exper. M., 1913, xvii, 232.
34. Warthin: J. Infect. Dis., 1916, xix, 138.
35. Nichols: J. Exper. M., 1914, xix, 362.
36. Levaditi and Marie: Ann. de l'Inst. Pasteur, 1919, xxxiii, 741. *Ibid.*, 1923, xxxvii, 189.
37. Fournier and Schwartz: Ann. de l'Inst. Pasteur, 1923, xxxvii, 183.
38. Wassermann, Neisser, and Bruck: Deutsche med. Wchnschr., 1906, xxxii, 449.
    Wassermann, Neisser, Bruck, and Schucht: Ztschr. f. Hyg., 1906, lv, 451.
39. Noguchi: J. Am. M. Ass., 1912, lviii, 1163.
40. Craig and Nichols: J. Exper. M., 1912, xvi, 336.
41. Zinsser: Textbook of Bacteriology. 1922, p. 318.
42. Walker and Swift: J. Exper. M., 1913, xviii, 75.


### TREPONEMA RIGIDUM Zinsser and Hopkins

Isolated by Zinsser and Hopkins from the tissues of five different strains of rabbit syphilis.

Very fine spiral with regular corkscrew curves and finely tapering ends, varying in length from two to ten or twenty rather shallow curves. Both long and short forms are two to three times as thick as *Treponema pallidum* and a definite double contour is visible in most individuals. Average length one-half to three to four times that of *Treponema pallidum* with occasionally very much longer forms. Organisms absolutely rigid and non-motile. Stains with great difficulty. Contours faint and often less distinct than those of *Treponema pallidum* after twelve hours' staining in dilute Giemsa. Demonstrated by Loeffler's flagella stain and by Fontana's method. Not stained by ordinary dyes. Cultivated in ascitic agar tubes containing rabbit kidney (Noguchi method).

Zinsser and Hopkins: J. Bact., 1916, i, 489.

### TREPONEMA REFRINGENS Schaudinn and Hoffmann

Described originally as *Spirochæta refringens* by Schaudinn and Hoffmann.[1] It occurs in the smegma and in ulcerating lesions of the skin and has been found in syphilitic ulcers, but usually more in the superficial layers than in the deep. Possibly identical with the vibrio described by Donné[2] in 1837 in genital secretions in syphilitic individuals under the name *Vibrio lineola.*

**Morphology.**—Under dark field illumination *refringens* is not so sharply outlined as *pallidum*, and appears golden-yellow, while *pallidum* appears pale yellow. It is longer and thicker than *pallidum* and highly refractile when fresh. The turns of the spiral are fewer in number, of greater amplitude, less regular, and flattened. Park, Williams, and Krumwiede[3] state that *refringens* has three to fifteen irregular flat spirals which change shape when the organism is in motion and measures $\frac{1}{2}$ to $\frac{3}{4}$ $\mu$ in diameter and 10 to 30 $\mu$ in length. In cultures *refringens* measures 0.5 to 0.75 $\mu$ in diameter and 6 to 24 $\mu$ in length (Noguchi[4]). The spiral amplitude and the intervals are 2 to 4 $\mu$, usually more or less regular. Spiral depth 0.5 to 1 $\mu$. Number of spirals or waves varies from three to five. In live specimens the spiral depth is almost constant and the waves change position regularly. In stained specimens the organisms are often irregularly flattened out. Chains of 2, 3, or 4 individuals are frequently seen, connected to each other by a fine filament. The middle part of the body is wavy, while the extremities are more regularly and deeply curved. The organisms may also be regularly curved throughout the entire length, the distance between the two curves being about 2 $\mu$. Ends pointed, and to one or both is usually attached a fine long more or less stiff, but elastic projection which has regular minute curves. Body cylindrical. No undulating membrane. Multiplication by transverse division.

**Motility** active, more so than with *pallidum.* In the cultivated *refringens* the curved flagella-like projections rotate with extraordinary rapidity around their point of attachment. The body of the organism may remain motionless or be transversed by waves, convex curves becoming concave and concave curves becoming convex. The waves begin at one end and pass along the entire body or may stop after passing over a few curves.

**Stains** easily with ordinary dyes, bluish or violet-red by Giemsa (Noguchi).

Levaditi found that *refringens* could be cultivated in collodion sacs in the rabbit's peritoneum. Cultivated by Noguchi from a moist condyloma about the anus of a female patient, in a medium consisting of 1 part ascitic fluid and 2 parts of alkaline agar and a piece of rabbit's kidney. The tubes were incubated anaërobically at 37° C. and after two weeks an impure culture was obtained. *Treponema refringens* grows luxuriantly in the deeper part of the medium, forming hazy colonies which gradually extend to the upper part. Growth stops within 2 cm. of the surface. It is visible after about four days and then continues to increase for some weeks. No change occurs in the medium and no odor develops. Growth continues without the addition of fresh tissue. Like *microdentium*, the growth is poorer without tissue.

**Non-pathogenic** when inoculated into rabbits, testicles, or beneath the skin in *Macacus rhesus* (Noguchi). *Balanitis erosiva circinata* shows a large spirochete like *refringens* regarded by Prowazek and Hoffmann as the causative agent (*Treponema balanitidis*).

1. Schaudinn and Hoffmann: Arb. a. d. Kais. Gsndhtsamte, 1905, xxii, 527.
2. Donné: Recherches microscopique sur la nature des mucus et de la matiére des divers écoulements des organs génito-urinaires chez l'homme et chez la femme. Paris, 1837.
3. Park, Williams, and Krumwiede: Pathogenic Microörganisms. 1920, p. 560.
4. Noguchi: J. Exper. M., 1912, xv, 90. *Ibid.*, 1918, xxvii, 667.

### TREPONEMA PERTENUE (Castellani)

The etiological agent in yaws, parangi, or *frambœsea tropica*, was first reported by Castellani[1] as *Spirochæta pallidula*. Castellani thought that it might be identical with *Spirochæta pallida*. It was also called *Spirochæta pertenuis* by Castellani,[2] and is now usually referred to as *Treponema pertenue*. Castellani's observations were confirmed by vom dem Borne.[3]

**Morphology.**—Extremely delicate thin spirochete generally tapering at both ends. It varies in width from 0.3 to 0.5 $\mu$ and in length from a few microns to 18 to 20, and is marked by uniform small waves. Often 2 organisms are attached to each other. It usually has six to twenty spiral turns. Ashburn and Craig[4] report that yaws is fairly common in the Philippine Islands. They give the length in specimens stained by Wright or Giemsa as 10 to 12 $\mu$ for the usual forms, 4 $\mu$ for short forms, and 25 to 30 $\mu$ for long specimens. The width varies from $\frac{1}{6}$ to $\frac{1}{2}$ $\mu$ and is usually about $\frac{1}{4}$ $\mu$ (0.25 $\mu$). Length of spiral turn 1.5 $\mu$, measured from crest to crest. Curves fine, 1 to 1.2 $\mu$ in depth, regular and rather rigid. Division transverse.

Various aberrant forms have been described. Castellani and Prowazek[5] have observed round or oval corpuscles measuring 5 to 8 $\mu$ in length and 4 to 6 $\mu$ in breadth of unknown significance. Ashburn and Craig noted ring forms and organisms with dots or granules and knob-like ends. A terminal projection or process is sometimes but not always present.

**Motility** active lashing much like that of *pallidum*.

**Stains** best with Giemsa or Wright.

Constantly present in the papules and ulcers in yaws and also found in non-ulcerated lesions.

Not cultivated.

**Transmission to Animals.**—Yaws was first communicated to monkeys, *Semnopithecus priamus* and *Macacus pileatus*, by Castellani, who used material from yaws in man. A skin eruption developed confined to site of inoculation, and a general infection with the organism. *Treponema pertenue* was found in the spleen and lymphatic glands and the material was only infectious when it contained the spirochete. When the organism was removed by filtration the material ceased to be infectious. Positive results were obtained with blood from the general circulation and from the spleen of yaws patients. Monkeys developing yaws had no immunity to *Treponema pallida* and monkeys with syphilis had no immunity to yaws. Castellani demonstrated the Bordet-Gengou reaction of complement fixation with yaws antigen and antibody and showed that they are different from the syphilis antigen and antibody. According to Castellani the disease is usually transmitted by contact. Castellani's observations were subsequently confirmed by Levaditi and Nattan-Larrier,[6] who found *Treponema pertenue* in yaws and showed that it was impossible to transmit the disease to syphilitic monkeys. It had previously been shown by Neisser, Baermann, and Halberstädter[7] that there was a distinct difference in immunity between yaws and syphilis. Ashburn and Craig produced yaws in 5 monkeys, *Cynomolgus philippensis*. All developed typical lesions after an incubation period of twenty days, *Treponema pertenue* being found in them. In rabbit's testicles lesions like those of syphilis have been produced by Nichols.[8]

In man the disease may be differentiated from syphilis by the Wassermann reaction, which is not given by patients with yaws alone.

1. Castellani: Brit. M. J., 1905, ii, 1330.
———: Arch. f. Schiffs-u. Tropen-Hyg., 1907, xi, 19.
2. Castellani: J. f. Hyg., 1907, xii, 558.
3. vom dem Borne: J. Trop. M., 1907, x, 345.
4. Ashburn and Craig: Philippine J. Sc., Sect. B. Med. Sc., 1907, ii, 441.
5. Prowazek: Arb. a. d. K. Gsndhtsamte, 1907, xxvi, 23.
6. Levaditi and Nattan-Larrier: Ann. de l'Inst. Pasteur, 1908, xxii, 260.
7. Neisser, Baermann, and Halberstädter: München. med. Wchnschr., 1906, xxxviii.
8. Nichols: J. Exper. M., 1910, xii, 616. *Ibid.*, 1911, xiv, 196.

### TREPONEMA OBTUSUM (Castellani)

Found by Castellani[1] in the open lesions of "*frambœsia tropica*" and named *Spirochæta tenuis obtusa*.[2] Thin delicate organism with a variable number of coils of different size. Blunt ends. Stains faintly purplish or bluish by Leishman's alcoholic solution.

1. Castellani: Brit. M. J., 1915, ii, 1280.
2. Castellani: Brit. M. J., 1905, ii, 1330.

### TREPONEMA ACUMINATUM (Castellani)

Found in the open lesions of *"frambœsia tropica"* by Castellani[1] and named *Spirochæta tenuis acuminata.*[2] Thin delicate wavy organism tapering at both ends. Stains faintly purplish or bluish by Leishman's alcoholic solution.

1. Castellani: Brit. M. J., 1905, ii, 1280.
2. Castellani: Brit. M. J., 1905, ii, 1330.

### TREPONEMA ABORIGINALE (Cleland)

Described as *Spirochæta aboriginalis* by Cleland[1] and obtained by him from ulcerative granuloma of the pudenda in the aborigines in Australia. Stained in the tissues by Cleland.

Typical spiral organisms somewhat thicker than *Treponema pallidum*, with less numerous coils lacking the corkscrew appearance. They measure from 7.91 to 15 $\mu$ in length, average 10.73, and have two to five coils (Cleland). Bosanquet,[2] who studied the organisms from Cleland's material, states that when stained by Levaditi they are very small, 12 $\mu$ in length, and so slender that they can hardly be measured. They might be $\frac{1}{4}$ $\mu$ in width. Short forms, 3 to 4 $\mu$ in length, also common. Some organisms thicker, $\frac{1}{2}$ $\mu$ in breadth. Some forms granular, the majority homogeneous. The granules may be greater in diameter than the

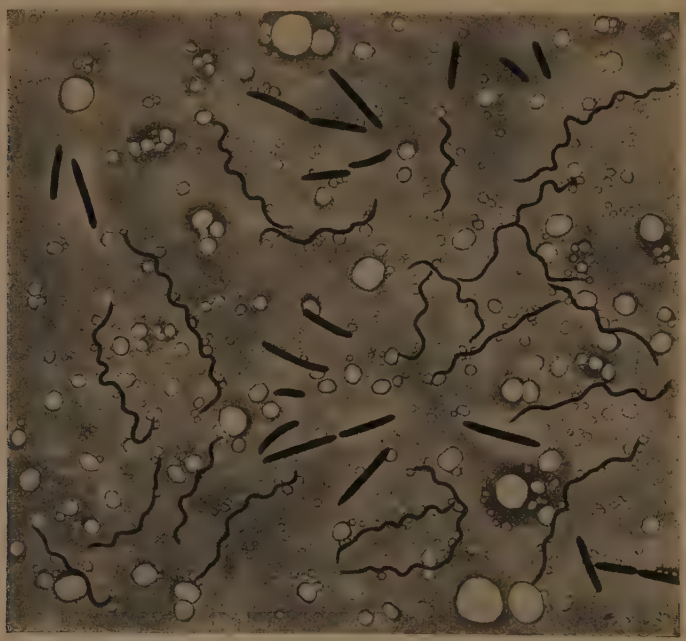

Fig. 182.—Film from tropical ulcer, showing spirochetes and long bacilli, stained with carbol-fuchsin.

rest of the organism. Terminal granules not uncommon. A few forms occur with two granules at one end. Sometimes the whole spirochete looks like a string of beads. Forms also occur with large terminal granules, transparent, and unstained, as well as thick specimens with well-marked loops near one end. A few coiled into rings. Curves irregular, not like those of *pallidum*. Some spirochetes almost straight, some twisted, some merely sinuous.

*Treponema aboriginale* looks a little like *pertenue*.

1. Cleland: J. Trop. Med. and Hyg., 1909, xii, 143.
2. Bosanquet: Parasitology, 1909, ii, 344.

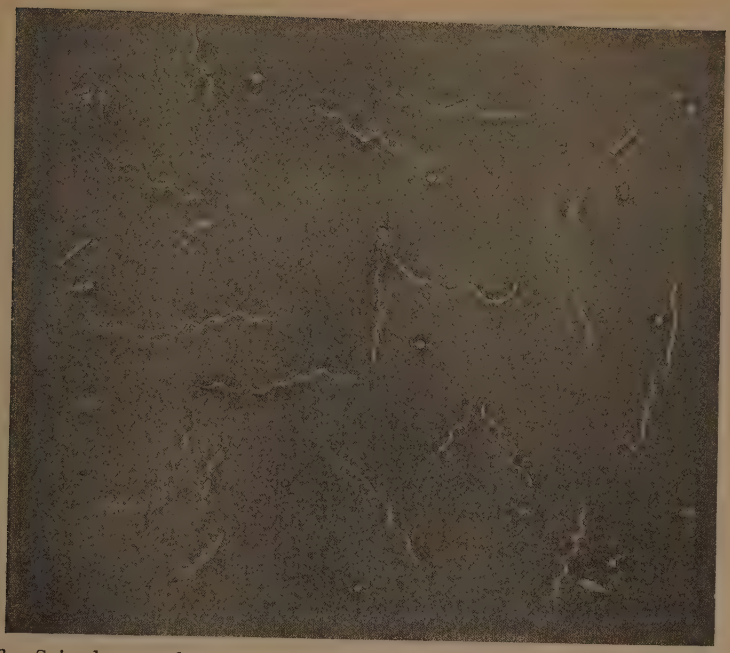

Fig. 183.—Spirochetes and small organisms from mouth, as seen in dark field illumination.

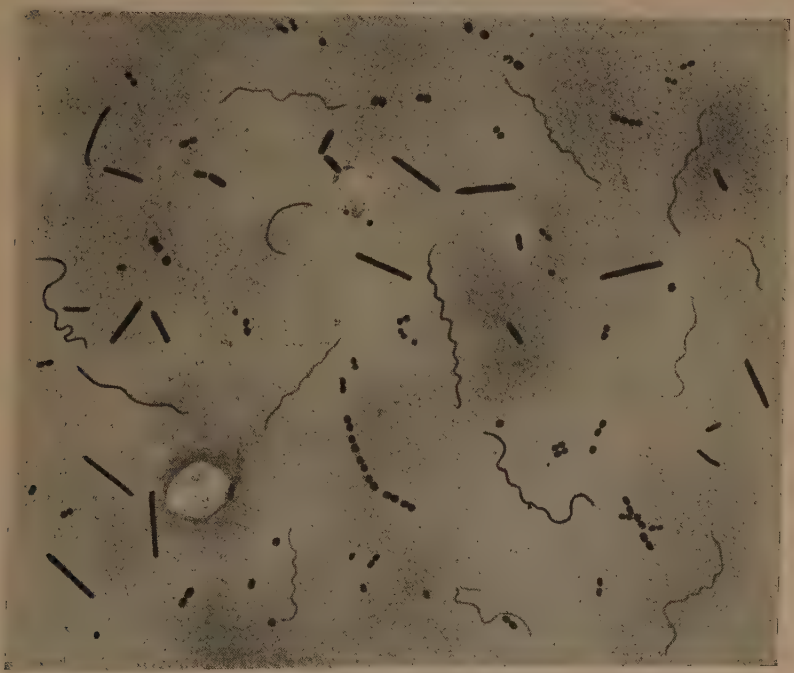

Fig. 184.—Spirochetes from saliva, mixed with long bacilli and streptococci.

### TREPONEMA BUCCALE (Lühe) Dobell

Reported by Cohn[1] as a mouth spirochete and apparently named *Spirochæta buccalis* by Lühe.[2] Dobell[3] states that the term "buccalis" was introduced by Steinberg. Described by Dobell as *Treponema buccale.* It is the largest of the mouth spirochetes and is almost constantly present in normal individuals.

It varies in length from 7 to 20 $\mu$ and has a small and variable number of turns to the spiral. Thickness approximately 0.3 $\mu$. Dobell finds that the real size is not obtained in dried specimens stained with Giemsa or Loeffler which make the organism appear too large, but in organisms fixed in osmic acid. By Heidenhain's stain the thickness varies from 0.3 to 0.4 $\mu$, by Loeffler's flagella stain from 0.4 to 0.9 $\mu$. Ends sharply pointed in properly preserved organisms, but appear rounded and clubbed after drying and staining with Giemsa, due to artefacts. Body circular in transverse optical section, not flat or ribbon-like.

**Motility** active. The organisms have considerable flexibility which gives a serpentine rather than a screw-like motility. Movements consist of translation, rotation, and flexion, very often performed simultaneously. Mechanism of motility not yet explained clearly. Flagella have not been definitely demonstrated. The long-drawn out ends or "periplastic processes" are not flagella, but may be the attenuated extremities of newly divided individuals. No anteroposterior polarity. No undulating membrane. Internal structure lacking (Dobell). Chromatin bodies have been described, but they are probably due to artefacts. No metachromatic granules.

**Stains** violet by Giemsa. By Heidenhain shows granules or transverse bands probably artefacts.

**Multiplication** by transverse fission. This may be observed in the living organism or may be demonstrated in fixed and stained preparations. A faint break first appears in the middle of the body, this widens and appears as a constriction, then a long-drawn-out connecting filament develops, which later snaps. The newly formed ends are sharply pointed after division of the parent organism. Division resembles the drawing out of a glass rod in the flame and takes place in about five minutes. Motility persists during division. No incurvation as in the cristispiras. No longitudinal division. The organisms vary in length from 7 $\mu$ to more than twice that, the thickness remaining constant. This is in conformity with transverse division, not with longitudinal division.

Plasmolysable in 5 or 10 per cent. NaCl (Swellengrebel,[4] Dobell). The organisms apparently have a cell membrane like that of the bacteria. No change in appearance when treated with saturated solution of sodium bicarbonate. Solutions of potassium, 10 to 20 per cent., make the organisms brighter. Not affected by 5 per cent. nitric acid.

Not cultivated in pure culture. In impure culture *buccale* resembles *refringens.*

According to Kolle and Hetsch[5] there are two subspecies of *Treponema buccale, undulatum* and *inequale,* and two varieties, *tenue* and *rectum.* Hoffmann makes three species of *Spirochæta buccalis, crassa, tenuis,* and *inequalis.* Noguchi[6] suggests that *buccale* may be a spironema and not a treponema.

1. Cohn: Beitr. z. Biol. d. Pflanz., 1875, i, Heft 3, 199.
2. Lühe: *In:* Handb. d. Tropenkrankh. (Meise). Leipzig, 1906.
3. Dobell: Arch. f. Protistenk., 1912, xxvi, 117.
———: Quart. J. Micr. Sc., 1911, lvi, 507.
4. Swellengrebel: Ann. de l'Inst. Pasteur, 1907, xxi, 573.
5. Kolle and Hetsch: 5. ed., p. 854.
6. Noguchi: J. Exper. M., 1912, xv, 80.

### TREPONEMA DENTIUM (Lühe) Dobell

Observed originally by Cohn and by Koch[1] as "Spirochete des Zahnschleims" and apparently first named *Spirochæta dentium* Koch by Lühe,[2] the term "dentium" coming from Miller. There are several different varieties of small spirochetes about the teeth which have received different names, and the nomenclature is very confusing. Thus Kolle and Hetsch[3] divide *Spirochæta dentium* into the true *Spirochæta dentium* and *Spirochæta denticola.* Hoffmann[4] describes *Spirochæta dentium* (*denticola*) as the most common and names it also *Spirochæta orthodonta.* He likewise describes *Spirochæta skoliodonta* and *Spirochæta trimerodonta,* the last like the spirochetes in Weil's disease, *Spirochæta icterohemorrhagiæ,* for which he makes a

new name *Spirochæta trimeres*. The teeth spirochetes are the smallest of the mouth spirochetes and may be differentiated by their size from the organisms known as *Spirochæta buccalis* or *Treponema buccale*. An intermediate form between *dentium* and *buccale* has been seen by Hoffmann and Prowazek.[5] *Spirochæta dentium* was definitely named *Treponema dentium* by Dobell.[6]

*Treponema dentium* varies in length from 4 to 18 μ, average length being 10 μ (Dobell). Short forms, 3 μ long, are occasionally found, but are infrequent. Thickness fairly constant, approximately 0.2 μ. Body cylindrical, not ribbon-like. Spiral more regular in living forms than in *Treponema buccale*. Ends sharply pointed. Clubbed or round ends seen in fixed preparations, not during life. No nuclear material. Internal structure uniform. Turns of spiral closer together and not so deep as in *pallidum* (Besson). From one to twelve spirals.

**Division** transverse, not longitudinal. This may be demonstrated in living organisms. No incurvation.

**Motility** active. Flagella not definitely demonstrated. No undulatory membrane.

**Stains** pink by Giemsa, black by Heidenhain.

Teeth spirochetes were first cultivated by Mühlens[7] in weakly alkaline or neutral horse serum agar (2 parts agar, 1 part serum), heated to 58° to 60° C., boiled to drive off the oxygen, and incubated anaërobically at 37° C. In the first generation colonies appeared in eight to ten days as fine homogeneous vapor-like masses. When older they showed dark yellow centers, non-granular, without outgrowths. They were ½ to 5 mm. in size and looked like colonies of the organisms of swine-erysipelas or mouse septicemia. Transfers to stab cultures in serum agar gave a growth along the stab in about three days as a cloudy turbidity with many thick yellowish or brownish areas. Often fine cloudy separate colonies appeared like a string of pearls. After five to six days the whole tube of medium became milky and the line of growth disappeared. Colonies always several centimeters below the superficial surface, the organisms being strict anaërobes. No liquefaction of the media. Foul penetrating odor of protein decomposition.

Cultivated also occasionally on ascites fluid agar and on sheep, calf, rabbit, or goat serum agar. Growth also on sugar serum agar. No gas production. No growth on media devoid of animal proteins. No growth at room temperature. Vitality retained for three to four weeks at 37° C., ten to fifteen days at 22° C., and several days on ice, occasionally forty-three days at 37° C.

**Non-pathogenic** for mice and guinea-pigs.

Park, Williams, and Krumwiede[8] report that *Treponema dentium* can be cultivated on poured serum agar plates inoculated with material from the mouth and incubated at 37° C. under anaerobic conditions. After nine to twelve days colonies appear in the plates and may be transferred to agar stabs.

1. Koch: *In:* Cohn's Beitr. z. Biol. d. Pflanz., 1877, ii, 421.
2. Lühe: Handb. d. Tropenhrankh. (Mense). Leipzig, 1906.
3. Kolle and Hetsch: 5. ed., p. 854.
4. Hoffmann: Deutsche med. Wchnschr., Jahrg., 1920, xl, i, 253.
5. Hoffmann and Prowazek: Centralbl. f. Bakteriol. [etc.], 1906, xli, 741; 817.
———: Prowazek: Arb. a. d. K. Gsndhtsamte, 1907, xxvi, 23.
6. Dobell: Arch. f. Protistenk., 1912, xxvi, 117.
7. Mühlens: Deutsche med. Wchnschr., 1906, xxxii, 797.
———: Ztschr. f. Hyg., 1906, lv, 81.
8. Park, Williams, and Krumwiede: 1920, p. 559.

### TREPONEMA INTERMEDIUM Dobell

Spirochætæ or treponemata intermediate in size between *Treponema buccale* and *Treponema dentium* were first noted by Hoffmann and Prowazek[1] and by Prowazek.[2] This species was named *Treponema intermedium* by Dobell in 1912.[3] Dobell describes it as almost identical with *Treponema buccale*, but slightly smaller. Length 6 to 14 μ. Breadth 0 to 25 μ. Ends more rounded than in *Treponema buccale*. Spirals looser and more irregular. Motility like that in *buccale*. No internal structure. Plasmolysis in 5 to 10 per cent. NaCl. Division transverse. Dobell thinks this may be the species cultivated by Rapaci.[4]

1. Hoffmann and Prowazek: Centralbl. f. Bakteriol. [etc.], 1906, xli, 741; 817.
2. Prowazek: Arb. a. d. K. Gsndhtsamte, 1907, xxvi, 23.
3. Dobell: Arch. f. Protistenk., 1912, xxvi, 117.
4. Rapaci: Compt. rend. Soc. de biol., 1911, lxx, 784.

### Treponema microdentium Noguchi

Described by Noguchi as a mouth treponema probably identical with some of the forms included under *Spirochæta dentium* or *Treponema dentium*. It was cultivated by him especially from the teeth of children.

**Morphology.**—Organisms with wide convolutions, in cultures less than 0.25 $\mu$ in thickness at the middle of the body, gradually tapering toward both extremities which are sharply pointed. Young cultures show short forms. Old cultures may reach 8 $\mu$ in length and show fourteen curves. Active rotatory motion. Body flexible. Thin flagella-like processes at one or both ends. Other forms are long with regular almost rectangular curves; these may be a different species. With incomplete occlusion of oxygen from the cultures many irregular, wavy forms are seen like those found in smears from deposits between the teeth.

**Stains** red by Giemsa.

**Cultivated** in sheep-serum-water containing a piece of sterile tissue, testicle, or kidney of rabbit or sheep, covered with a layer of paraffin oil and incubated at 37° C. Mixed cultures of spirochetes and bacteria were first found. Pure cultures were obtained in wide tall tubes of serum agar (1 to 3) containing sterile tissue, inoculated by stab. Hazy colonies of spirochetes grew out along the stab, the canal of which was filled with colonies of bacteria. From these hazy colonies transfers were made to fresh serum agar stabs. A faint haze appeared along the stab in seventy-two hours at 37° C. This increased in intensity and colonies developed in the agar and in the space between the agar and the wall of the tube. Young cultures have a faint odor. Old cultures have a characteristic strong fetid odor. In fluid cultures the serum is coagulated, and litmus, when added, decolorized.

Injection into testicles and over the eye of a *Macacus rhesus* caused an induration. No spirochetes found in it.

Noguchi: J. Exper. M., 1912, xv, 81.

### Treponema macrodentium Noguchi

Observed by Noguchi and cultivated by him, especially from cases of Vincent's angina or ulcerative stomatitis. It may be the same as Vincent's spirochete, but this has not been ascertained with certainty. According to Noguchi this form is like the medium spirochete of Hoffmann and Prowazek (*Treponema intermedium* of Dobell).

**Morphology.**—Somewhat similar to *pallidum*, but with larger and less regular spirals. In young cultures plump, with short, irregular, shallow curves. Extremities taper off abruptly. Under dark field illumination bodies of organisms show double refraction. Lashing lateral motility or vibration. Organisms flexible and capable of creeping through the agar medium in all directions. Long delicate flagella-like processes are attached to one or both ends. From older cultures the spirochetes are longer and thinner and taper more gradually toward the ends, the curves are almost rectangular, shallow, and quite regular, and the motility is lost. Young organisms measure 0.7 to 1 $\mu$ in width by 3 to 8 $\mu$ in length and have two to eight curves. Older specimens measure 0.3 $\mu$ in width, 12 $\mu$ or more in length, and have fourteen or more curves. In fluid media the organisms are long and wavy. When oxygen is not completely occluded irregular non-typical forms appear.

Stains more intensely with the blue of Giemsa than *pallidum*, appearing red only in poorly prepared specimens.

**Cultivated** in the media employed for *Treponema microdentium*, sheep-serum-water containing a bit of sterile tissue, and serum agar containing sterile tissue.

Noguchi: J. Exper. M., 1912, xv, 81.

### Treponema gracile (Veszprémi)

Named originally *Spirochæta graciliis* by Veszprémi and found by him in gangrenous inflammations.

Usually about 9 to 12 $\mu$ long, with fine, regular spirals varying from 6 to 12. Very fine and thin and so pale on staining that the organisms are recognized with difficulty. Ends pointed.

Stains paler than the ordinary bacteria, best with dilute Ziehl's carbolfuchsin. Stains also by Gram if the solution is boiled. Not stained by ordinary Gram or Weigert in sections. In tissues stains best with carbolfuchsin or with Levaditi's silver method.

No evidence of nucleus or chromatin. No involution-like granules or vacuoles, etc., in sense of bacterial involution.

Cultivated with other bacteria only, the organisms appearing late, three to seven days after inoculation.

This organism may be allied to Noguchi's *Treponema macrodentium.*

Veszprémi: Centralbl. f. Bakteriol. [etc.], 1907, xliv, 332; 408; 515; 648.

### TREPONEMA MUCOSUM Noguchi

Cultivated from the pus in *pyorrhea alveolaris* by Noguchi.

**Morphology.**—Resembles *Treponema pallidum* and *Treponema microdentium* in size and shape, and measures 0.25 to 0.3 $\mu$ in thickness by 8 to 12 $\mu$ in length. The curves are regular and deep and vary in number from 6 to 8. The extremities are sharply pointed and often possess at one or both ends fine minutely curved projections, varying in length in different organisms. These projections may be 8 to 10 $\mu$ in length. The organisms are often joined to each other by a thin filament forming a pair or chains of 3, 4, or more individuals. When cultivated under favorable conditions peculiar irregular forms occur, some straight and some slightly curved. Many granular particles are seen which may be degeneration products or segments which are capable of reproducing the organism under favorable conditions. These segments take the chromatin stain and vary in size. Sometimes long treponemata are found undergoing granular segmentation which may be degeneration, and sometimes short forms are seen attached to round bodies as if they had sprouted from them.

**Motility** graceful, rotatory.

**Staining Reactions.**—The same as with *Treponema microdentium.* The organisms take the red in the Giemsa dye.

**Cultivation** accomplished by Noguchi in solid media consisting of 1 part of ascitic fluid, 2 parts of ordinary agar with a piece of sterile rabbit kidney at the bottom, the whole medium being covered with paraffin oil. The tubes are incubated at 37° C. for ten days. The growth is whitish and semitransparent and spreads out from the line of inoculation which is densely crowded with bacteria. Pure cultures are made by transferring this whitish semi-transparent material to fresh tubes.

In pure cultures colonies become visible in twenty-four to forty-eight hours and render the agar unevenly opalescent in the depths up to 1.5 cm. of the top. The individual colonies are dense whitish turbidities with no definite outer boundaries. In thickly seeded and rapidly growing cultures the colonies unite and render the agar quite opalescent. The growth is denser than that of *microdentium.* No gas formed. Strong fetid, odor given off from the cultures. After two to three weeks the tissue becomes grayish and then quite dark. The culture now contains a varying amount of mucin. On breaking up the agar the mucin appears as extremely fine strings of filaments stretching from one piece of agar to another. It is not sticky, and according to Noguchi is not like the mucins produced by bacteria.

The organisms remain alive for several weeks at 37° C. The capacity to produce mucin is lost after repeated transfers (sixteenth generation one hundred and sixty days), but the capacity to produce a bad odor in cultures is retained.

*Treponema mucosum* is an anaërobe. It grows also without tissue and in fluid serum which is rendered opalescent without coagulation. A very strong odor is produced in fluid media.

**Pathogenic Action.**—Injection of a large quantity of active fluid culture into the cutaneous tissue of a *Macacus rhesus* monkey produces an acute inflammation, the tissues remaining indurated for a week or ten days. After twenty-four hours the tissue contains no living organisms and there is no tendency to suppurate. In the testicles of rabbits the organisms produce an acute inflammatory induration lasting about a week, but no abscess formation. The condition returns to normal within about two weeks. If the testicle be inoculated with an emulsion of an agar culture it becomes intensely inflamed in twenty-four hours and remains so for ten days. The inflammation gradually subsides, leaving a hard, circumscribed nodule for many weeks. On opening the testicle a yellowish, thick pus pours out. This has a fetid odor and contains the organism in small numbers. Pure cultures can be obtained from this pus. It thus appears that *Treponema mucosum* can survive in the testicle of a rabbit only when foreign bodies are introduced with the organisms.

Differentiated from *Treponema microdentium* by the denser growth, the mucin, the fetid

odor, and survival in rabbit's testicle. Not distinguishable morphologically. The *pallidum* differs from it by its pathogenic action, its lack of odor or mucin, and its requirement of fresh tissue in the culture-media.

Noguchi: J. Exper. M., 1912, xvi, 194.

### TREPONEMA CALLIGYRUM Noguchi

Cultivated by Noguchi[1] from condylomata in 2 cases, one syphilitic and one not syphilitic.

**Morphology.**—The majority of the organisms appear short and vigorously motile in solid cultures three to four days old, while in cultures two weeks old they are longer and less active. In fluid media both short and long forms are present from the beginning, varying in length and thickness. In pure cultures the organisms measure 0.35 to 0.4 $\mu$ in width by 6 to 14 $\mu$ in length. Forms as short as 4 $\mu$ and as long as 30 $\mu$ may occur. The curves are very regular and quite deep. The distance between the curves is about 1.6 $\mu$ and the depth of a curve about 1 to 1.5 $\mu$. The apex of the curves is not angular, but more or less rounded. Spirals five to eight, varying according to spiral amplitude, sometimes only three (Noguchi[2]). Occasionally the curves are shallower and the organisms become almost straight, probably due to artificial cultivation. The width is uniform except at the terminal portion, which ends in a sharp point. The organism often shows a delicate, regularly curved, rather stiff projection attached to one or both ends. The length varies, but it usually measures about 6 $\mu$. This projection appears oftenest in fluid cultures about six weeks old. Body cylindrical.

**Motility** active, chiefly rotatory, sometimes undulating, seldom lashing. Lateral vibration like that in *macrodentium*, *Obermeieri*, and *gallinarum*, not observed in *calligyrum*.

*Division* transverse.

**Staining Reactions.**—Stains reddish-violet by Giemsa, faintly red by carbolfuchsin. Decolorizes by Gram's stain.

Cultivated by Noguchi in a mixture of 1 part of ascitic fluid and 2 parts of slightly alkaline agar with a piece of fresh, sterile tissue at the bottom of the tube. The medium is covered with a layer of paraffin oil and the tubes are incubated at 37° C. At the end of ten days the line of inoculation is heavily crowded with colonies of bacteria, while a hazy zone radiates from this central core up to within 3 cm. of the surface. This hazy zone is made up of the treponemata. Pure cultures can be obtained by transferring some of the material from this hazy growth to fresh tubes. In three to four days, hazy opalescent colonies appear about the tissue and gradually spread toward the upper surface. At the end of four weeks the growth extends to within 2 cm. of the surface, but does not get any higher. Colonies are diffuse and more dense than those of *pallidum*. The organism also grows well in fluid media containing ascitic fluid in the method devised for *pallidum*. The medium does not change and no odor develops. The spirochetes grow in ascitic agar without the addition of fresh sterile tissue, but not so well as with tissue. Strict anaërobe. No growth at room temperature.

**Non-pathogenic.**—No lesions produced in the skin of monkey (*Macacus rhesus*, *Cercopithecus callitrichus*, *Papio fabuan*) or in the testicle or cornea of rabbits.

Differentiated from *microdentium* and *mucosum* without difficulty, as these are much smaller and produce an unpleasant odor in cultures. Much thicker than ordinary average or thinner type of *pallidum* in culture, but when thin may be confused with the thicker type of *pallidum*. This is nevertheless thinner than *calligyrum*. The majority of the *pallidum* are about 0.3 $\mu$ in width, the majority of *calligyrum* 0.4 to 0.45 $\mu$ in width even under unfavorable conditions. In suitable solid media the thick *pallidum* never exceeds 0.3 $\mu$, while *calligyrum* measures 0.35 to 0.4 $\mu$. The curves of the *pallidum* are more rigid and closely set. This organism grows without fresh tissue, the *pallidum* never. Much thinner than *balanitidis*, which has a width of 0.5 to 0.75 $\mu$.

Same as *macrodentium* in length, but thinner, has a tapering body, irregular curves, and creeping and lateral vibratory movements. *Macrodentium*, moreover, always requires tissue for cultivation.

Probably the same as the organism described by Levaditi and Stanesco,[3] which they called *gracilis*, but which was not identified by them with *gracilis* of Veszprémi.[4]

1. Noguchi: J. Exper. M., 1913, xvii, 89.
2. Noguchi: J. Exper. M., 1918, xxvii, 667.
3. Levaditi and Stanesco: Compt. rend. Soc. de biol., 1909, lxx, 188.
4. Veszprémi: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1907, xliv, Orig., 332.

### TREPONEMA GRACILE Levaditi and Stanesco

Described originally as *Spirochæta gracilis* by Levaditi and Stanesco[1] and cultivated from a chancre and from a case of balanitis.

Slightly thicker than *pallidum* when stained by Giemsa and by Loeffler. Undulations more regular and narrow than those of *pallidum*. Ends fringed or tapering. Motility more active than that of *pallidum*. Stains blue to rose-red by Giemsa in about thirty minutes. *Pallidum* takes red color more slowly. Cultivated in mixed cultures in collodion sacs in tubes of horse serum and in horse or human serum heated to 75° C. in the peritoneal cavity of a rabbit or monkey (Schereschewsky's method[2]). Slightly pathogenic. When inoculated into the prepuce of a chimpanzee it produces an erosive lesion like balanitis, appearing in forty-seven hours and healing spontaneously.

1. Levaditi and Stanesco: Compt. rend. Soc. de biol., 1909, lxx, 188.
2. Schereschewsky: Deutsche med. Wchnschr., 1909, xxxv, i, 835.

### TREPONEMA MINUTUM Noguchi

A minute treponema found by Noguchi[1] in the male, and by Noguchi and Kaliski[2] in the female genitalia.

Varies from 3 to 14 $\mu$ in length, with an average length of 7 to 10 $\mu$. Thickness 0.25 to 0.3 $\mu$. Spiral amplitude 0.9 to 1 $\mu$ with fairly regular intervals. Spiral depth 0.2 to 0.5 $\mu$ with some reaching 1 $\mu$ in penultimate spirals. Spirals vary in number from 7 to 10, the number varying with the length of the organism. *Treponema minutum* is decidedly smaller than *Treponema pallidum* and has a larger number of shallower spirals in proportion to its length.

Cultivated anaërobically at 37° C. in ascitic fluid media containing a piece of fresh tissue, and in solid media, 2 parts of neutral agar to 1 part of ascitic fluid, also containing tissue. No change in color in the fluid media, but the solid medium shows a faint haze near the tissue, gradually extending upward. No discrete colonies. No putrefactive or offensive odor.

Actively motile in young cultures.

Not *Treponema minutum* of Castellani which was found in rhinopharyngitis by Castellani in 1916.[3] This organism has very few spirals, varies in length from 3 to 12 $\mu$ and stains pinkish-red with Romanowsky. Not *Treponema minutum* of Dobell.

1. Noguchi: J. Exper. M., 1918, xxvii, 667.
2. Noguchi and Kaliski: J. Exper. M., 1918, xxviii, 559.
3. Castellani and Chalmers: 3. ed., London, 1919, p. 450.

### TREPONEMA PHAGYDENIS (Noguchi)

Described by Noguchi[1] as *Spirochæta phagydenis* in 1912 from a slowly progressing phagedenic ulcer on the external genitalia of a woman.

**Morphology.**—Slowly motile irregularly wavy spiral organisms in the original purulent material. Length 4 to 30 $\mu$, usually about 15. Width about 0.75 $\mu$. Some organisms almost straight, the majority having one to eight spiral turns. Often in the form of an interrogation point. Ends definitely pointed, but not sharply drawn out. The short organisms bend slowly to a semicircle and then stretch out suddenly with a whipping motion. Occasionally the spirochete moves the terminal portion to the left or to the right as an earthworm moves its head. In pure cultures the majority of the forms measure 10 to 15 $\mu$ and show only one or two waves. In old cultures they are 20 to 30 $\mu$ long with more distinct and numerous very irregular waves. The width is 0.7 to 0.8 $\mu$, not always uniform, sometimes nodular swellings and constrictions appearing in the middle or in each third of the body. Ends fairly pointed, but may be obtuse. Young forms are frequently straight, but may bend their bodies into parabolic curves and then straighten out again. These forms have a sluggish forward movement like that of a creeping worm. The terminal portion of the body is quite flexible and is moved like a feeler. No flagellum, terminal projection, or undulating membrane. In old cultures the organisms are granular and eventually disintegrate, and spherical bodies 1.5 $\mu$ in diameter appear. These may be attached to the ends or sides of short forms still well preserved. Under certain undetermined cultural conditions during disintegration numerous round or ovoid bodies are produced varying in size. Some are as large as 3 $\mu$ in width. They may be single, in pairs, or clusters. Highly refractile dots appear at one side of the protoplasmic mass in these bodies. Two such dots are visible in the larger forms. Forms are also found with more or less irregular refraction in different parts of the spiral body.

**Staining Reactions.**—This organism is difficult to stain with anilin dyes. It does not stain by Gram. It stains red with Giemsa's solution. The round bodies do not take ordinary spore stains and with Giemsa show a small dot of chromatin material at one pole. The highly refractile dots mentioned above take a deep red stain with Giemsa. The irregular refractive areas in the spiral body take a deep color with nuclear dyes.

**Cultivated** by Noguchi in ascitic agar containing bits of tissue covered with paraffin oil and incubated at 37° C. At the end of a week a faint haze appears along the side of the stab which is filled with colonies of bacteria. This suggests the appearance of a culture of *pallidum*. The hazy growth increases for two to four weeks and is made up of treponemata. Pure cultures obtained by transferring material from this hazy growth to new tubes. In these a similar hazy growth appears about the tissue in forty-eight hours and gradually extends upward. No growth at 15° C. Growth only as an anaërobe. Fresh tissue required. No visible alteration of the tissue or ascitic fluid. A distinct, putrefactive sour odor somewhat resembling butyric acid develops in the cultures.

**Pathogenic Action.**—Produces an acute inflammation lasting about three days without ulceration when introduced intradermally in *Macacus rhesus* monkeys and in rabbits. In rabbit's testicles it causes a temporary induration, disappearing completely in forty-eight hours.

**Differentiation.**—Not the same as *refringens* of Schaudinn and Hoffmann,[2] *balanitidis* of Prowazek and Hoffmann,[3] *pseudopallidum* of Mulzer.[4] Possibly the same as Polland's[5] large spirochete with five to six blunt curves, or Róna's *Spirochæta gangrænosæ nosocomialis*.[6]

1. Noguchi: J. Exper. M., 1912, xvi, 261.
2. Schaudinn and Hoffmann: Arb. a. d. K. Gsndhtsamte, 1905, xxii, 527.
   ———: Berl. klin. Wchnschr., 1905, xlii, 673.
3. Prowazek and Hoffmann: Centralbl. f. Bakteriol. [etc.], 1906, xli, Orig., 741; 817.
4. Mulzer: Berl. klin. Wchnschr., 1905, xlii, 1144.
5. Polland: Wien. klin. Wchnschr., 1905, xviii, 1236.
6. Róna: Verhandl d. deutsch. dermat. Gesellsch., 1907, ix, 471.

### TREPONEMA BALANITIDIS (Hoffmann and Prowazek)

Discovered by Simon[1] in balanitis and described as a motile spirillum like that found in recurrent fever. Spiral forms also regularly noted by Berdal and Bataille[2] in a special form of infectious balanitis described by them as *Balanitis erosiva circinata*. Studied by Hoffmann and Prowazek[3] and named by them *Spirochæta balanitidis*.

**Morphology.**—Rather strongly refractile, spiral organism, shorter and thicker than *pallidum*, with six to eight coils. Hoffmann and Prowazek described a ribbon-like body and an undulating membrane, but this was not confirmed.

**Motility** active, the spirochetes moving forward and backward with a screw-like movement. Also lateral sinuous movements are seen. After long pauses irregular, abrupt waves move over the whole body as refractile points.

**Stains** by Loeffler's flagella stain or Giemsa's eosin-azur, after fixation by osmic acid vapor by the method of Weidenreich.[4] Also stains by Heidenhain's iron hematoxylin. Bluish-red by Giemsa. With neutral red the periplast stains pale yellow-red. Cilia-like processes appear on both ends by Loeffler's flagella stain, periplastic extensions which show curves or spirals like the organism. When short they resemble the cilia of bacteria. No plasmolysis in 10 per cent. NaCl. Dissolved in two hours in dilute caustic. Dies in distilled water and glycerin.

**Cultivated** by Levaditi and Stanesco[5] in fluid horse serum or human serum, heated to 75° C., in collodion sacs in the peritoneal cavity of a rabbit. Never obtained in pure culture.

Mühlens[6] cultivated an organism found in *Balanitis erosiva circinata* by Müller and Scherber[7] up to the fourth generation. It was a strict anaërobe growing on serum-containing media on sixth to eighth day after inoculation. It formed cloudy colonies in agar, thicker and larger than those of teeth spirochetes, giving off a disagreeable odor. Mühlens regards this organism as the same as *refringens*.

**Pathogenic Action.**—Slight lesions produced in *Macacus hecki* and *Cercocebus fuliginosus* by inoculating them with pus from *Balanitis erosiva circinata*. The spirochetes appeared in the lesions which healed rapidly.

1. Simon: Tr. Internat. Cong. med., London, 1881, iii, 138.
2. Berdal and Bataille: Méd. Mod., 1891, 340.
3. Hoffmann and Prowazek: Centralbl. f. Bakteriol. [etc.], 1906, xli, Orig., 741; 817.
4. Weidenreich: Folia hæmatol., 1906, iii, 1.
5. Levaditi and Stanesco: Comp. rend. Soc. de biol., 1909, lxx, 188.
6. Mühlens: Ztschr. f. Hyg., 1906, lv, 81.
7. Müller and Scherber: Arch. f. Dermat. u. Syph., 1905, lxxvii, 77.

### TREPONEMA VINCENTI (Blanchard)

Spiral organisms were first reported in a peculiar ulcerative stomatitis by Plaut[1] and by Vincent,[2] and this condition has since been described as Plaut-Vincent's angina or as Vincent's angina. Both authors observed spindle-shaped or fusiform bacilli and spirochetes like those in recurrent fever.

The bacilli are non-motile straight or curved rods about 0.5 to 0.8 $\mu$ in width and 3 to 10 $\mu$ in length, tapering toward the ends. They are readily stained by Loeffler's methylene-blue and Giemsa, generally decolorize by Gram, and show great inequality in staining, bands or stripes running across the organism. They may resemble the diphtheria bacillus. The spirochetes are longer than the bacilli and are made up of shallow, irregular undulations,

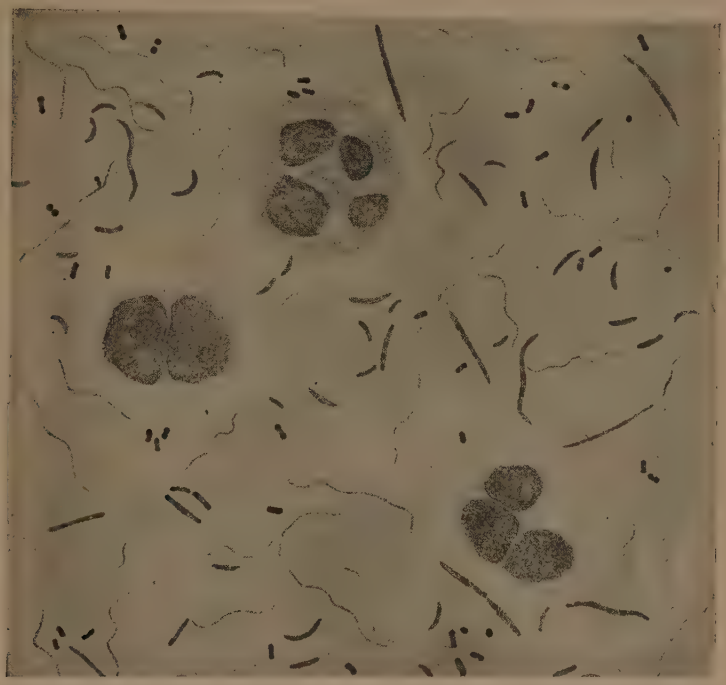

Fig. 185.—Spirochetes and fusiform bacilli from Vincent's angina, stained with safranin.

which are not so steep as in *Treponema pallidum*. They stain with difficulty, but are regular and homogeneous. Mixed cultures of fusiform bacilli and spirochetes have been obtained by Tunnicliff[3] on ascitic agar slants incubated anaërobically at 37° C. The bacilli appeared during the first five days, then died out, and the spirilla appeared. Animal inoculation with this material was without result. Fusiform bacilli and spirilla have been found by Weaver and Tunnicliff in noma, and by Gros[4] in cases of gingivitis among the Kobyles. Fusiform bacilli have now been grown free from spirilla by a number of authors, but thus far the spirilla have not been definitely cultivated. (See Fusiformis Vincenti.)

1. Plaut: Deutsche med. Wchnschr., 1894, xx, 920.
2. Vincent: Ann. de l'Inst. Pasteur, 1896, x, 488.
———: Bull. et mém. Soc. Méd. d. Hôp. de Par., 1898.
3. Tunnicliff: J. Infect. Dis., 1906, iii, 148.
4. Gros: Bull. Soc. path. exot., 1909, ii, 282.

### TREPONEMA BRONCHIALE (Castellani)

Spiral organisms were originally found by Castellani[1] in cases of bronchitis in Ceylon and named by him *Spirochæta bronchialis*. Bronchial spirochetosis

has since been recorded by Chalmers and O'Farrell[2] in the Anglo-Egyptian Soudan, by Violle[3] in cases of bronchitis originating in the tropics, by Peters, Bloedorn, and Houghton[4] in America, by Faust[5] in China, the organisms observed being like Castellani's spirochete. Lancereaux[6] has reported a pneumothorax with a bloody fluid containing spirochetes in large numbers and Mason[7] has found Castellani's organism in a spontaneous infection of the pleural cavity resulting in an empyema with a foul, fetid fluid. The life history of this spirochete has been especially investigated by Fantham.[8]

**Morphology.**—The organisms vary greatly in size, long and thick spirochetes occurring with short and thin forms. Length varies from 5 to 27 $\mu$, the usual forms measuring about 8 $\mu$, many about 15 $\mu$. Width varies from 0.2 to 0.6 $\mu$. Ends tapering, pointed, or roundish. Cytoplasm homogeneous, chromatin bars showing at intervals along the organism (Fantham). These give rise to the chambered or alveolar appearance emphasized by Gross.[9] Number of coils variable. Division transverse. Narrow thin membrane or crista described by Fantham as a lateral outgrowth of the periplast.

**Motility** very rapid, organisms moving forward and turning on the long axis at the same time. Movement double, undulating flexion of the body and corkscrew movement taking place at the same time. The treponemata may curve the ends inward like a watch spring, and then straighten out, intercoil their ends, and seem to tie themselves in knots.

**Stained** easily by Giemsa and Leishman, carbolthionin, gentian-violet, and by various hematoxylins, Delafield's, Ehrlich's, and Böhmer's, best results being obtained by overnight exposure to weak solutions. Best fixed by osmic vapor followed by absolute alcohol, by Schaudinn's fluid (sublimate-alcohol-acetic-acid), and by Bouin's fluid (picro-formol-alcohol-acetic acid).

**Granule Phase.**—Fantham thinks that the chromatin bars, which appear as minute, refractile granules, have formed about them a concentration of cytoplasm. These concentrations gradually become oval, the outer cytoplasmic layer differentiates as a thin coat, and ultimately a series of coccoid bodies or granules is formed, lying transversely or obliquely within the peripheral sheath of the organism. The sheath ruptures or disintegrates and sets free these coccoid bodies. They elongate and very small spirochetes emerge from them. Fantham believes that these coccoid bodies may transmit the infection. This granule phase has not thus far been definitely proved.

Not cultivated.

**Pathogenic Action.**—Chalmers and O'Farrell have produced an anterior mediastinitis in monkeys, with cough and mucous expectoration, by intratracheal injection of material containing these organisms. The sputum of the inoculated animals contained many spirochetes. Other authors, like Mason, have had negative or equivocal results with animals. In man the sputum harbors many spirochetes during the bronchitis, but they disappear rapidly after recovery sets in.

1. Castellani: Lancet, 1906, i, 1384.
———: Brit. M. J., 1909, ii, 782.
2. Chalmers and O'Farrell: J. Trop. M. and Hyg., 1913, xvi, 329.
3. Violle: Rev. gén. de clin. et de thérap., 1918, xxxii, 144.
4. Bloedorn and Houghton: J. Am. M. Ass., 1921, lxxvi, 1559.
5. Faust: Arch. Int. Med., 1922, xxx, 343.
6. Lancereaux: Presse méd., 1909, xxvii, 556.
7. Mason: Johns Hopkins Hosp. Bull., 1920, xxxi, 435.
8. Fantham: Ann. Trop. M. and Parasitol., 1915, ix, 391.
9. Gross: Mitt. a. d. Zoöl. Stat. zu Neopel, 1920, xxi, 41.
———: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1912, lxv, Orig., 83.

### Nolf and Spehl's treponema

Nolf and Spehl[1] have reported bronchitis and bronchopneumonia due to spirochetes in soldiers in the Belgian Army who have never been in the tropics. The breath and expectoration become fetid and a few or many spirochetes are found in the sputum which is not usually hemorrhagic. They are different from Castellani's spirochetes.

These organisms stain badly with ordinary basic anilin dyes, best with Fontana's mordant or by Spehl's method[2] (mordant of formol and chromic acid, stain formolized violet, reinforced by Lugol). By Fontana the spirals are brownish black, by Spehl, bluish-black. Length 8 to 12 $\mu$. About as thick as Vincent's spirochete. Ends tapering. No flagella. Con-

volutions short, sometimes regular, sometimes irregular, seven to eight for adult form rarely, ten to eleven.

1. Nolf and Spehl: Arch. Méd. belges, 1918, lxxi, 1.
   ———: Nolf: Arch. Int. Med., 1920, xxv, 429.
2. Spehl: Compt. rend. Soc. de biol., 1918, lxxxi, 305.

### TREPONEMA SCHAUDINNI (Prowazek)

Spirochetes in tropical ulcers have been known since early time. They were observed early by Neisser in Java. According to Lühe,[1] Smith and Peill, in Sierra Leone, and Patton, in Aden, found spirochetes in these ulcers like the spiral organisms in carcinomas. They were seen by Prowazek[2] in tropical ulcers along with fusiform bacilli and pyogenic cocci and named *Spirochæta Schaudinni.*

**Morphology.**—Long, wavy spiral organisms and short plump simply wound individuals. Thinner than the organisms found in balanitis by Prowazek and Hoffmann.[3] Motility like that of the latter. Periplastic terminal extensions only on one end (Prowazek), fine, resembling the flagella of bacteria. Prowazek described the cell body as ribbon-like and surrounded by a very fine refractile membrane, but this has not been confirmed. Terminal swellings appear on or near the end of the organisms, at the same time the spirochetes tend to roll up, form spirals, and wind themselves in skeins. These swellings have a concentrated protoplasm, stain intensely, and remain attached to the organisms. Prowazek regards them as resting stages. Prowazek has claimed a longitudinal division which has not been verified. Dissolved in sodium taurocholate (1 : 10). Not stained by neutral red in the dark. In the sunlight the spirochetes are sensitized by neutral red, lose motility, and in certain cells large reddish-black granules appear. Taken up by polymorphonuclear leukocytes, eventually losing their motility and staining light red.

Non-pathogenic to *Macacus cynomolgus* or to the orang-outang. Not cultivated.

1. Lühe: Handb. der Tropenkrankh. (Mense).
2. Prowazek: Arb. a. d. K. Gsndhtsamte, 1907, xxii, 23.
3. Prowazek and Hoffmann: Centralbl. f. Bakteriol. [etc.], 1906, xli, 741; 817.

### TREPONEMA EURYGYRATUM (Werner)

Described originally by Werner[1] in his own normal dejecta. Le Dantec[2] previously mentioned a spirochete in the dejecta, 7 to 14 $\mu$ long, occurring in three forms, wavy with 3 turns, spiral form and buckled. Mühlens[3] mentions small and large spirochetes in the dejecta. This form of Werner has wide curves. Length 4.6 to 7.3 $\mu$. Body flexible. Spiral movement, organisms looking like a banner waving in the wind. Turns or curves very wide, not more than two in number, so that S forms are common. Often collected in waving streamers about masses of protoplasm, just as Le Dantec describes for his form. This form resembles Le Dantec's.

Cultivated by Hogue[4] in Locke egg, ovomucoid, ox-bile salts media, and in a special medium consisting of 15 c.c. of 0.85 sodium chlorid solution and 0.3 sterile serum water, with a $pH_7$ reaction, tubes covered with paraffin oil. Growth aërobic. Hogue's[4] organisms were obtained from a case of chronic diarrhea. They were stained by iron hematoxylin, Giemsa, weak carbolfuchsin, and by Cross's method. Length 4 to 56 $\mu$. Division by transverse fission and incurvation. Motility active, darting, wave-like, and undulatory. Body flexible. Non-pathogenic to cats. The coccoid bodies described by Porter as a means of transmission were not found by Hogue.

1. Werner: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1909, lii, Orig., 241.
2. Le Dantec: Précis de pathologie exotique, Paris. 1905, p. 667.
3. Mühlens: Ztschr. f. Hyg., 1907, lvii, 405.
4. Hogue: J. Exper. M., 1922, xxxvi, 617.

### TREPONEMA STENOGYRATUM (Werner)

Found by Werner in his own normal dejecta. Spirochetes with narrow spirals.

Length 3.5 to 6.1 μ. Curves or spirals vary from two to six. Length of spiral usually 1.3 μ. Height of spiral 0.58 μ. Less flexible than *eurygyrata*. A sinuous or wavy motion of the body with a change in form of the spiral turns, with a screw-like forward and backward motion, can easily be made out. Longitudinal division described by Werner, but not verified. Button-like enlargements are not uncommon, looking as if the last spirals were wound together. Resembles *Spirochæta dentium* in morphology somewhat, but the latter is narrower, longer, and has more spirals.

Werner: Centralbl. f. Bakteriol. [etc.], 1 Abt., 1909, lii, Orig., 241.

### TREPONEMA CUNICULI (Jacobsthal) Noguchi

A venereal disease of rabbits was first described by Ross[1] and organisms in it, like *Treponema pallidum*, were originally noted by Bayon.[2] They were also found by Arzt and Kerl,[3] who transmitted the infection to rabbits. Spiral organisms were observed in this disease by Jacobsthal,[4] who named them *Spirochæta paralues cuniculi*. The disease was first investigated in American rabbits by Noguchi,[5] who named the organisms *Treponema cuniculi*. The infection is characterized by ulcerative lesions of the genitalia both in the male and female. Spontaneous rabbit syphilis also observed by Neumann.[6]

**Morphology.**—The organisms look like *Treponema pallidum*. They measure 0.25 μ in width by 10 to 16 μ in length. Spiral amplitude 1 to 1.2 μ. Spiral depth 0.6 to 1 μ. The number of spirals in individuals measuring 10 μ in length is about 9 or 10. Ends sharply drawn out and a terminal filament at one or both ends may be made out. Shortest forms with six spirals measure 7 μ in length. Longest forms with twenty-five to twenty-eight spirals measure 30 μ. Average length 12 to 14 μ. Organisms slightly longer than *Treponema pallidum* in lesions.

**Stains** like *pallidum*, with ordinary basic anilin dyes when fixed in a buffered solution of formaldehyd (Tilden's method[7]).

Disease transmitted experimentally by Noguchi to rabbits, not to monkeys. Incubation period five to eighty-eight days. No Wassermann reaction. Cured by salvarsan.

Noguchi found a coarser organism, in addition, in the lesions of this disease like *Treponema calligyrum*, but a trifle longer and thinner.

1. Ross: Brit. M. J. 1912, ii, 1653.
2. Bayon: Brit. M. J., 1913, ii, 1159.
3. Arzt and Kerl: Wien. klin. Wchnschr., 1914, xxvii, 1053.
4. Jacobsthal: Dermat. Woch., 1920, lxxi, 569.
5. Noguchi: J. Exper. M., 1922, xxxv, 391.
6. Neumann: Centralbl. f. Bakteriol. [etc.], 1923, xc, 100.
7. Noguchi: J. Am. M. Ass., 1921, lxxvii, 2052.

### TREPONEMA BUFONIS (Dobell)

Found once by Dobell[1] in the rectum of *Bufo vulgaris* in very small numbers. Described as *Spironema bufonis* and *Spirochæta bufonis*.

It measures 8 to 10 μ in length by about 0.5 μ in width. Very few turns to the spiral, which displays some irregularities owing to the flexibility of the organism. Average distance between the turns about 3 μ. Resembles *Spirochæta buccalis* of Cohn. No terminal flagella demonstrated. Very actively motile. Protoplasm shows no internal structure, but a granular appearance like that figured by Jaffé[2] for *Spirochæta culicis* from Culex larvæ.

On two occasions Dobell observed another actively motile treponema in the large intestine of *Bufo vulgaris*. It measured 5 to 12 μ in length, about 0.2 μ in width, and resembled *Treponema pallidum*.

1. Dobell: Quart. J. Micr. Sc., 1908, lii, 121
2. Jaffé: Arch. f. Protistenk., 1907, xi, 100.

### TREPONEMA MINUTUM Dobell

Noted originally by Dobell[1] as possibly a treponema, named more definitely later (Dobell[2]). Occurs as a very rare parasite in the large intes-

tine of the toad, *Bufo vulgaris*. Very small, thickness constant, in organisms fixed by the wet method (sublimate alcohol) and stained with Heidenhain approximately 0.2 $\mu$. Some evidence of transverse division. No evidence of longitudinal division.

1. Dobell: Quart. J. Micr. Sc., 1908, lii, 121.
2. Dobell: Arch. f. Protistenk., 1912, xxvi, 117; Quart. J. Micr. Sc., 1909, liii, 279.

### TREPONEMA STYLOPYGÆ Dobell

Found by Dobell in the hind-gut of the common cockroach, *Stylopyga orientalis*. Found in only a small percentage of cockroaches, and only those from Cambridge, not in cockroaches in London.

Length 3 to 4 $\mu$ to about 11 $\mu$, measured across the turns of the spiral. Average length of newly divided organisms about 5 to 6 $\mu$, shorter individuals being uncommon. Breadth about 0.3 $\mu$. Considerable variation in form. The organisms may show many close turns in the spiral, a few loose turns, or an irregular spiral of a varying number of turns may be seen. Rolled-up forms not uncommon. General resemblance to *Treponema buccale*, but ends are blunt, not pointed. No crista or flagella. Body appears circular on cross-section. Actively motile like other treponema. No anteroposterior polarity.

In dried Giemsa preparations the organisms are sometimes seen to possess swollen ends, one or both ends appearing clubbed or globular. Living or properly fixed organisms do not present these appearances which Dobell believes are brought about by drying. Stains red or pink with Giemsa, black or gray with Heidenhain, according to the degree of differentiation. No internal structure made out.

Transverse division conclusively demonstrated in three consecutive stages. A break first appears in the middle of the organism, the two daughter individuals subsequently drawing apart at this point. They remain attached for a short time by a very fine connecting strand. Division like that in *Treponema termitis*. No evidence of longitudinal division.

Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

### TREPONEMA PARVUM Dobell

Seen on two occasions in the hind gut of the common cockroach, *Stylopyga orientalis*, by Dobell. Exceedingly uncommon and excessively small.

In general forms it resembles *Treponema dentium*, and is a very slender and fairly regular though flexible spiral. Maximum length about 7 $\mu$. Width about 0.2 $\mu$ or less, Movements very active and difficult to follow. No internal structure made out in living or stained individuals. Some evidence of transverse division.

Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

### TREPONEMA TERMITIS (Leidy)

Spiral organisms were originally described by Leidy[1] in the North American termites, *Reticulitermes flavipes*, and called first a *spirillum*, then a *vibrio*. They have been recorded in the European termites, *Kalotermes flavicollis* and *Reticulitermes lucifugus*, by Grassi and Sandias.[2] Observed especially by Dobell[3] in the gut of the Ceylon termite, *Kalotermes militaris*. Named *Vibrio termitis* by Leidy and *Spirochæta termitis* (Leidy pro parte) Dobell by the latter author. Dobell thinks that the organisms originally described as spirilla were probably spirochetes or treponemata. Dobell found two spiral organisms in the Ceylon termite, the larger of which he identifies with Leidy's spirillum or vibrio. His description also applies to Prowazek's *Treponema minei*.

*Treponema termitis* was the largest treponema known to Dobell, 20 to 60 $\mu$ in length, about 0.5 $\mu$ wide in wet films stained by Heidenhain's method. Sharply pointed at both ends. Circular in optical transverse section.

**Motility** very active, organisms darting rapidly in the direction of long axis. No antero-posterior polarity. Movements like those of other treponemata except on a larger scale. Rolling up and vibratory movements also seen. No crusta. No flagella. Spiral form more marked in living organism than in fixed and stained preparation. The latter may appear almost straight.

Internal structure chambered. Chambers distinctly visible in some organisms fixed (wet) in picro-acetic solution (3.1) and stained with Heidenhain's iron hematoxylin. In individuals dried and fixed in absolute alcohol and stained by Giemsa short "chromatin" rods and spirals appear, due to preparation plasmolysis. These are apparently caused by breaking down of the chambered structure just as in *Cristispira*.

*Division* must take place by transverse fission, since the width remains constant, only the length varying. This mode of division was visible in fixed and stained organisms, not in living spirochetes. Longest individuals show very frequently a well-marked break in the middle of the body, at first an almost imperceptible pale transverse line. A constriction appears later at this point, two approximately equal daughter spirochetes being formed. Seen best in organisms deeply stained, as with picro-acetic Heidenhain. Similar stages in division also seen in dried Giemsa preparations. Stains uniformly pink by Giemsa.

A number of different termites, from Porto Rico, British Guinea, Florida, California, Oregon, and Maryland have recently been examined for spirochetes by Damon.[4] They were found in ten different species and varied greatly in size and abundance.

1. Leidy: Proc. Acad. Nat. Sc., Philadelphia, 1877, xxix, 146.
———: Jour. Acad. Nat. Sc., Philadelphia, 1881, viii, 441.
2. Grassi and Sandias: The Constitution and Development of the Society of Termites, etc., 1893–94. Translation in Quart. J. Micr. Sc., 1893–94, xxxix, 245.
3. Dobell: Spoliana Zeylanica, 1910–13, vii, 80.
———: Quart. J. Micr. Sc., 1911, lvi, 507.
4. Damon: J. Bact., 1926, xi, 31.

### TREPONEMA MINEI (Prowazek) Dobell

Noted first by Prowazek[1] as *Spirochæta minei* from the Japanese termite, *Termites lucifugus*. Subsequently investigated and named by Dobell,[2] who believes that it is practically the same as *Treponema termitis* except smaller.

Longest individuals about 25 $\mu$ in length; newly divided forms or still attached daughter individuals measure on an average about 10 $\mu$. Shorter individuals also seen, about 6 $\mu$ in length. Number of turns to the spiral varies considerably, the organisms being very flexible and often presenting a very irregular appearance in stained preparations. Breadth about 0.3 $\mu$. No chambered structure recognized by Dobell with certainty, but in dried alcohol-fixed Giemsa specimens preparation plasmolysis gives rise to appearances like those seen in *Treponema termitis*, so a kind of chambered structure probably exists. Multiplication by transverse fission into two individuals by the same process as in *Treponema termitis*. No longitudinal division. Ends pointed. Stains red or red-violet by Giemsa. Star-shaped rosettes and rolled-up forms noted by Prowazek.

1. Prowazek: Arch. f. Shiffs-u. Trop-Hyg., 1910, xiv, 297.
2. Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

### TREPONEMA GRASSI Doflein

Recorded by Doflein in European termites. Spirochetes measuring 100 $\mu$ in length, 1.5 $\mu$ in width, with wriggling and creeping movements. Chambered structure. Rudimentary crista.

Doflein: Problem der Protistenk. II. Die Natur der Spirochæten. Jena, 1911.

### TREPONEMA GLOSSINÆ (Novy and Knapp)

Obtained originally by Novy and Knapp[1] from the stomach contents of tsetse flies, *Glossina palpalis*, and named by them *Spirochæta glossinæ*.

The organisms occur as single short forms, 8 $\mu$ in length, and as double cells, 15 $\mu$ in length. They are shorter, narrower, and have more turns that *Spironema Obermeieri*. Width of filament 0.2 $\mu$, number of turns four, distance of turns 1.3 $\mu$. Width of turn or spiral 0.6 $\mu$.

The spiral organisms described by the Sargents[2] in the gut of the larva of *Anopheles maculipennis* closely resemble Novy and Knapp's spirochetes. They measured 8.5 to 17 $\mu$ in length and showed 1.5 to 4 turns. The spirochetes described by Macfie[3] in the gut of *Glossina technoides*, which measured 15 to 35 $\mu$ in length and 0.7 to 1 $\mu$ in width, are not the same.

1. Novy and Knapp: J. Infect. Dis., 1906, iii, 291.
2. Sargents: Compt. rend. Soc. de biol., 1906, x, 90.
3. Macfie: Ann. Trop. M. and Parasitol., 1914, viii, 439.

### TREPONEMA CTENOCEPHALI (Patton)

Noted by Patton as *Spirochæta ctenocephali*, parasitic in the alimentary tract of the Indian dog flea, *Ctenocephalus felis*. Found once in the adult insect taken from a dog and twice in the larvæ bred from fleas living on cats. These larvæ live almost exclusively on the excreta of the adult insect. The spirochete is not itself regarded as a parasite of the dog or cat. Seen only once in 500 fleas and twice in 1500 larvæ.

Motility active. In long forms, about 20 $\mu$ in length, eight to ten well-marked spirals appear, in short forms, 4 to 6 $\mu$ in length, about four spirals. Long forms divide transversely. The long forms may be coiled up toward the centers or at one of the ends, simulating the formation of cysts or spores. A faint pink band is seen at the concavities of the spirals and was first interpreted as an undulating membrane. Chromatic granules were made out in some of the shorter forms.

Patton: J. Trop. M. and Parasitol., 1912, vi, 357.

### TREPONEMA CULICIS (Jaffé)

Described as *Spirochæta culicis* by Jaffé[1] from the stomach of Culex larvæ. Appears as a spiral organism with regular spiral turns of about the same length. Screw-like motility backward and forward, the spirochetes retaining their stiff contour. Also a wave-like motion runs over the whole body in both directions. Completely dissolved in 1 per cent. potassium hydrate.

**Stains** by Giemsa after fixation with alcohol or osmic acid. Spirals vary from two to six in stained preparations. Also rolled up or knotted organisms appear. Whole body stains an even violet-red with Giemsa, with occasional clear areas or holes. These also show with Heidenhain's iron hematoxylin. With Giemsa deeply stained bodies show in the homogeneous light protoplasm. With Zettnow's flagella stain the organisms may appear yellowish-gray, filled with irregularly distributed particles of various sizes. No flagella by Zettnow's or Loeffler's stain. After maceration in distilled water and treating with Loeffler a deeply stained axial portion appears, surrounded by a lightly stained edge or seam. Occasionally this axial filament is inside a more or less broken and disintegrated peripheral seam.

Jaffé does not decide whether the deeply stained axial portion of the cell is to be regarded as an axial "Kernfaden" which Perrin[2] described for *Spirochæta Balbianii*, whether one can correlate it genetically with the "Randfaden" of the undulating membrane of the trypanosomes, or whether the lightly stained zone can be named "ectoplasm" or "periplast." The only conclusion he draws is that the spirochete consists of two different substances (Elementen), one which swells with difficulty and one surrounding this which easily swells. Giemsa's stain reveals the same type of attached or adjacent lighter seam. Method of division not clearly made out.

**Motility** active. The body of the organism stretches out and moves forward, the number of the spirals diminishing and their depth lessening. This may be compared with the stretching of a spiral spring. The body is flexible, changing suddenly to a closed circle, with the spirals disappearing, and then reverting to its regular form. This is especially the case when one end is fixed. Then the other end makes lashing movements, not unlike those of bacterial flagella.

1. Jaffé: Arch. f. Protistenk., 1907, ix, 100.
2. Perrin: Arch. f. Protistenk., 1906, vii, 131.

### TREPONEMA HARTMANNI (Gonder)

Described as *Spirochæta Hartmanni* by Gonder from stomach, intestine, and crystalline stile of the mussel, *Pinna squamosa* and *Pinna nobilis.*

Measures 6 to 14 $\mu$ in length and about 1 $\mu$ in thickness. Motility active, corkscrew and thrashing. Both ends finely pointed, the terminations being extensions of the periplast. A structure resembling an undulating membrane was visible in living organisms; in stained preparations it appeared as a narrow edge or seam. The number of spiral turns conforms to the size of the organism.

Stains by Giemsa or hematoxylin. Fine chromatin granules are present in the plasma and a nuclear rod (Kernstab) often arises from the periplast; this can be well recognized on the two ends. Longitudinal division described by Gonder, but not confirmed. This organism belongs in the group of *pallidum*, *dentium*, and *medium.*

Gonder: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1908, xlvii, 491.

### TREPONEMA VIVAX Dobell

Noted by Dobell as a free-living treponema from fresh water like the organisms from fresh water described by Doflein (1911). Found in water from river Cam at Cambridge.

**Morphology** varies greatly. Body exceedingly flexible and may have the form of a very definite spiral or lie almost straight. Spiral may be quite close with many turns (six or more) or loose with few turns (one or two). Organism moves with great rapidity and changes its shape continually. Movements like those of *Treponema buccale.* When moving slowly it may display bending, looping, and rolling-up movements like those of a small nematode. No anteroposterior polarity. Length 5 to 11 $\mu$, measured from end to end across the turns of the spiral. Width about 0.2 $\mu$. Living organisms colorless. No internal structure demonstrable. After fixation and staining with Heidenhain the organism appears a uniform gray. Red by Giemsa. No granules in cytoplasm. Division transverse, like that in *Treponema termiitis*, as evident from fixed and stained specimens. No longitudinal division. No crista. No flagella.

Dobell: Arch. f. Protistenk., 1912, xxvi, 117.

## 6. GENUS LEPTOSPIRA Noguchi

Type species *Leptospira icterohemorrhagiæ* (Inada, etc.) Noguchi.

Pathogenic and non-pathogenic varieties. Length 7 to 9 to 14 $\mu$, exceptionally 30 to 40 $\mu$. Pointed ends. Diameter 0.25 to 0.3 $\mu$. Body cylindrical. Spiral amplitude 0.45 to 0.5 $\mu$, regular, rigid. Spiral depth 0.3 $\mu$, regular. One or more gentle wavy curves throughout the entire length. When in free state one or both ends may be semicircularly hooked. When in semisolid medium the organism appears serpentine, wavy, or bent. Flexibility striking. Axial filament not demonstrated. Terminal spiral filament not recognized. Well-developed highly motile end-piece in last 6 to 8 spirals. No chambered structure, membrane, crista, or flagella. Division transverse. Membrane not demonstrable by stains. Body stains reddish-violet by Giemsa. Easily dissolved in bile salts (10 per cent.). Completely resistant to saponin (10 per cent.).

Type species *Leptospira icterohemorrhagiæ* (Inada, etc.) Noguchi.
" *biflexa* (Wolbach and Binger) Noguchi.
" *nodosa* (Heubner and Reiter).
" *icterogenes* (Uhlenhuth and Fromme).
" *icteroides* Noguchi.
" *hebdomadis* (Ido, Ito, and Wani) Noguchi.
" *aqueductum* (Uhlenhuth and Zuelzer).
" *salina* (Uhlenhuth and Zuelzer).
" *dentium* Hoffmann.
" *bovis* Noguchi.

**LEPTOSPIRA ICTEROHEMORRHAGIÆ (Inada, Ido, Hoki, Kaneko, and Ito) Noguchi**

Discovered in 1916 by Inada, Ido, Hoki, Kaneko, and Ito[1] in the blood, liver, kidneys, and adrenals in hemorrhagic jaundice, known also as epidemic jaundice, iceterohemorrhagic fever, and Weil's disease. Found also in the livers of guinea-pigs inoculated with blood from human cases. These animals developed albuminurea, jaundice, and hemorrhages when inoculated with blood from patients in the first seven days of illness. The jaundice in the guinea-pigs lasted six to thirteen days. The infection was subsequently kept alive in guinea-pigs for many generations. Described originally as a spirochete and subsequently made the basis of a new genus, *Leptospira*, by Noguchi.[2]

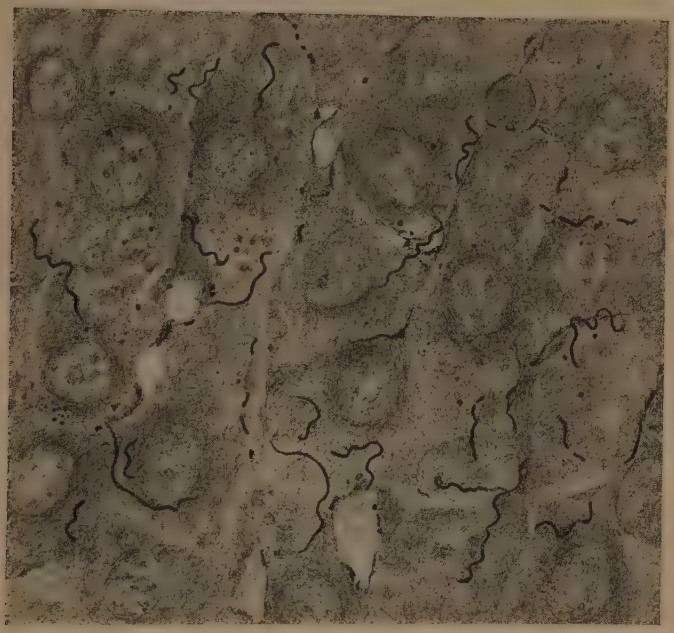

Fig. 186.—*Leptospira icterohemorrhagiæ*, film from infected guinea-pig.

**Morphology.**—Spiral organisms with irregular wavy undulations and small rectangular curves regularly set along the entire length. Length 6 to 15 *u*. The small rectangular curves vary in number from 2 to 3 to 15, depending on the length. The long specimens resemble *pallidum*. The body of the parasite is granular or beaded due to alternating more or less refractile granules. The average number of these is about 15. They are smaller toward the extremities which are drawn out into fine points. When resting, the ends of the spirochete may be blunt. Noguchi finds that this organism differs from other spirochetes in its minute elementary spirals and the remarkable flexibility of the terminal or caudal portion. It has no terminal flagellum. It resists the destructive action of 10 per cent. saponin, but is sensitive to bile and bile salts (Noguchi). Kaneko and Okuda[3] find that degenerative forms of the parasite occur in the human body, consisting of globoid granules, two to five times as thick as the spirochete, as well as ring forms and buds.

**Motility** active, forward, backward, and lateral. The organisms may shoot through the field with great rapidity or display corkscrew or serpentine movements along the whole axis.

**Staining Reactions.**—No staining with ordinary dyes. Organisms stain pink or purple with Giemsa. Stained specimens are heavier in the middle, tapering off into sharp points like *refringens*.

**Cultivated** by the discoverers of the organism by the Noguchi method, in deep tubes containing guinea-pig's blood instead of rabbit's blood, covered with paraffin oil. The fluid remains clear, the organisms living three to six weeks in the first generation and as long

as fifty-five days in the second. Cultivated by Ito and Matsuzaki[4] in guinea-pig's blood inoculated with blood or urine from cases of Weil's disease or from the liver or kidney of an inoculated animal. Grown also in blood agar (1 part guinea-pig or human blood + 1 to 2 parts agar) and in blood gelatin (1 part gelatin + 2 to 4 parts blood). The tubes may be covered with paraffin, but this is not necessary. Growth begins in about a week and continues two to three weeks. Growth obtained in fluid media, human or ox blood-serum diluted with an equal part of distilled water or with undiluted ascites fluid or pleural exudate sterilized at 50° to 60° C. for three days. Good growth in three to ten days. Cultivated by Noguchi[5] in serum diluted 1:10 by Ringer's solution or water, inoculated by citrated heart's blood. Fresh tissue not required. Grown in rabbit's serum diluted 1:10 with Ringer or water, with 0.5 to 1 part of neutral slightly alkaline 2 per cent. agar added. Growth is only at the surface. It is invisible in fluid media and produces a slight haze in semisolid media. According to Noguchi animal or human serum is necessary for the growth of this organism. The nutritive value of the serum is reduced by heating to 60° C. for thirty minutes and is destroyed by boiling. It is not diminished by filtration. Animal sera differ in value. Rabbit, horse, and goat sera are better than guinea-pig, sheep, donkey, or calf sera. Rat and pig sera are of no value. Ascitic fluid is not suitable. With rabbit's serum added to Ringer's solution a luxuriant growth is obtained when more than 10 per cent. serum is added; moderate growth occurs with 5 per cent. of the serum; no growth with less than 2 per cent. Nutritive substances necessary for the organism are not obtained from the organs of guinea-pigs or rabbits or from the white or yolk of eggs.

Best growth in slightly alkaline media, the alkalinity not exceeding that of serum. No growth in media made slightly alkaline by the addition of sodium hydroxid or slightly acid by hydrochloric acid. Carbohydrates have no influence and the reaction of medium containing them is not affected by the growth. Growth frankly aërobic. Grows at temperatures from 10° to 37° C. Optimum temperature 30° to 37° C. Growth more rapid at 37° C. than at 25° to 30° C. Cultures remain viable longer at the latter temperature. No growth at 42° C.

**Pathogenic Action.**—*Leptospira icterohemorrhagiæ* is virulent to guinea-pigs by intraperitoneal inoculation. The animals develop jaundice and die in four to eight days. At autopsy the leptospiras may be obtained from the urine and from the kidneys, rarely from the blood and other organs. Hemorrhages are more pronounced than in guinea-pigs infected with *Leptospira icteroides*.

In man the incubation period is five to seven days from skin infection (Inada[6]). The disease is not a relapsing fever, but an after-fever may occur. This happens in 28.2 per cent. of the cases and may be a reaction to the absorption of spirochetal toxins. The organisms are present in the urine (Ido, Ito, and Wani[7]). At autopsy the parasites are irregularly scattered in various regions of the body. They may be found in the blood, kidneys, adrenals, liver, spleen, pancreas, cardiac muscles, intestinal wall, prostate, testicles, and epididymis. They are not only in the blood, but inside the cells of the various organs.

Transmission to man apparently mechanical from the infected urine of rats or infected soil. Inada and Ido[8] believe that infection takes place chiefly through the alimentary canal, but may occur through abrasion of the skin. Ido, Hoki, Ito, and Wani[9] state that the disease may be conveyed from rats to guinea-pigs by the bite of the rat.

**Immunological.**—Guinea-pigs can be actively immunized to *Leptospira icterohemorrhagiæ* (Noguchi[10]). After three successive inoculations of 0.5 c.c. emulsions of killed cultures they become completely resistant to virulent cultures of homologous and heterologous strains. With smaller quantities protection is not so general. The active immunity was present eight weeks after the last inoculation and probably lasts much longer.

Immune serum has been employed somewhat in Japan for treatment, with possibly favorable results. Kaneko and Okuda report that the immune serum destroys the spirochetes in the organs with the possible exception of the kidney and has a spirocheticidal and spirochetolytic action.

**Distribution.**—*Leptospira icterohemorrhagiæ* has been isolated by Miyajima from the kidneys of field-mice and by Ido, Hoki, Ito, and Wani from house and roof rats, *Mus alexandrinus* and *Mus norvegicus*, in Japan. It has been found by Stokes, Ryle, and Tytler[11] in the kidneys of wild rats in Flanders, by Noguchi in wild rats in New York, by Jobling and Eggstein[12] in rats in Nashville, Tenn., by Ottereaen[13] in the rats about Chicago, by Robinson[14] and by Walch[15] in wild rats in Baltimore, by Noguchi[16] in wild rats in Guayaquil. Noguchi[17] reported that the Japanese, Belgian, and American strains are identical in general, but minute immunological differences may be made out. Thus two of the American strains form a group, one American strain and one European strain form another, and the Japanese strain represents a third group closely allied to the first. Another strain isolated in France by Manine, Cristan, and Plazy[18] is probably an unusually deviated type of this species. The organisms are present in the urine and in the kidneys of infected rats, not in the blood, mouth, or liver.

**Survival in Nature.**—Noguchi[19] finds that *Leptospira icterohemorrhagiæ* cannot grow in the urine because of its acidity, surviving less than twenty-four hours unless the urine is

made neutral or slightly alkaline. Then the survival is about ten days. It does not live in feces more than twenty-four hours unless they be autoclaved and filtered and have corpuscles and serum added. Under natural conditions of discharge the organism does not survive more than twenty-four hours in fecal material. Survival for only three days in polluted water, sewage, or soil. Aërobic bacteria like the typhoid, paratyphoid, and dysentery bacilli and the pneumococcus are antagonistic to it. Insects like mosquitoes, flies, and wood ticks do not become carriers when fed on infected guinea-pigs or their organs and cannot serve as the intermediate host.

1. Inada, Ido, Hoki, Kaneko, and Ito: J. Exper. M., 1916, xxiii, 377. *Ibid.*, 1917, xxv, 755.
2. Noguchi: J. Exper. M., 1918, xxvii, 575.
3. Kaneko and Okuda: J. Exper. M., 1917, xxvi, 325.
4. Ito and Matsuzaki: J. Exper. M., 1916, xxiii, 557.
5. Noguchi: J. Exper. M., 1917, xxv, 755. *Ibid.*, 1918, xxvii, 593.
6. Inada: J. Exper. M., 1917, xxvi, 355.
7. Ido, Ito, and Wani: J. Exper. M, 1918, xxviii, 435. *Ibid.*, 1919, xxix, 199.
8. Inada and Ido: J. Exper. M.., 1916, xxiv, 465.
9. Ido, Hoki, Ito, and Wani: J. Exper. M., 1917, xxvi, 341.
10. Noguchi: J. Exper. M., 1918, xxviii, 561.
11. Stokes, Ryle, and Tytler: Lancet, 1917, i, 142.
12. Jobling and Eggstein: J. Am. M. Ass., 1917, lxix, 1787.
13. Ottereaen: J. Infect. Dis., 1919, xxix, 485.
14. Robinson: Am. J. Hyg., 1924, iv, 327.
15. Walch: Some Observations on *Leptospira icterohemorrhagiæ* of the Wild Rats of Baltimore, Dissert. School of Hygiene and Public Health, Johns Hopkins University, 1925.
16. Noguchi: J. Exper. M., 1919, xxx, 95.
17. Noguchi: J. Exper. M., 1918, xxviii, 561.
18. Manine, Cristan, and Plazy: Compt. rend. Soc. de biol., 1917, lxxx, 531.
19. Noguchi: J. Exper. M., 1918, xxvii, 609.

### LEPTOSPIRA BIFLEXA (Wolbach and Binger) Noguchi

Described by Wolbach and Binger[1] as *Spirochæta biflexa* and as *Leptospira biflexa* by Noguchi.[2] Isolated from stagnant water from the shores of a fresh water pond near Boston by filtration through a Berkefeld V filter.

**Morphology.**—Small, actively motile organism easily seen under dark field illumination. Characterized by the extreme closeness of its turns, its small size, and its curved or flexed extremities. Average length 5 to 7 $\mu$. Amplitude or width of the spirals from crest to crest 0.2 to 0.25 $\mu$. The turns vary in number from 22 to 30. The ends are thinner than the bodies and taper to points, are more or less sharply curved, sometimes having the form of a crook. Rarely the bodies of the organism contain one or more chromatophilic granules. Occasionally very long forms occur and at times terminal enlargements.

*Motility.*—The organism spins with extreme rapidity on its long axis in such a way that the curved ends give the appearance of solid bulbs or flask-like bodies. A rapid translatory movement also occurs in either direction of the long axis. The living organism is straight and appears to have a rigid long axis.

Stained best by osmic acid and alcohol fixation followed by Giemsa. In stained preparations the bodies become bent or flexed and the spirals less regular and less closely wound.

This organism could not be cultivated on any media tried. It was kept alive for some time in the original tubes of filtered water.

1. Wolbach and Binger: J. M. Research, 1914, xxv, 23.
2. Noguchi: J. Exper. M., 1918, xxvii, 575.

### LEPTOSPIRA NODOSA (Huebner and Reiter)

The virus of Weil's disease in Germany was called *Spirochæta nodosa* by Huebner and Reiter. It was present in the peripheral blood in the first few days of the disease and could be transferred to guinea-pigs. These animals developed fever, emaciation, albumin and bile in the urine, nosebleed, bleeding from the conjunctiva, petechiæ in the skin, loosening of the hair, and an icterus of skin, mucous membranes, and sclera. With an intraperitoneal injection of 0.5 to 1 c.c. of blood from patients the guinea-pigs died in

five to twelve days. The virus also multiplies or stays alive in rabbits and monkeys as shown by the production of disease in guinea-pigs by transferring blood to them.

In the blood of infected guinea-pigs three to four hours after infection dark field examination shows small elongated forms with sinuous motility, only found after long observation of the blood. When stained by Giemsa the spirochetes appear red with a small terminal mass taking the red color. This gives a kind of tad-pole appearance. The parasites are often stretched out. They are present chiefly in the liver, rarely in the lungs, bone-marrow, and spleen. Demonstrated in the tissues by Levaditi's method. They frequently show knots, appearing in skeins or tangles.

Huebner and Reiter: Deutsche med. Wchnschr., 1915, xli, ii, 1275. *Ibid.*, 1916, xlii, i, 1.

### LEPTOSPIRA ICTEROGENES (Uhlenhuth and Fromme)

The virus of Weil's disease in Germany was also described by Uhlenhuth and Fromme[1] as *Spirochæta icterogenes*. The infection was transmitted to guinea-pigs by intraperitoneal injection of defibrinated blood. Fine delicate spirochetes were found in the blood, liver and other organs, bile and urine of the inoculated animals, and also in the liver and urine of patients. Blood of the patients was infectious in the first week and highly virulent, 0.001 c.c. of blood withdrawn just before exitus killing a guinea-pig. Infection was likewise produced by intracardial inoculation. The virus can pass through the scarified skin and the uninjured mucosa of the mouth. Rabbits are sensitive, but not hens, cats, dogs, shoats, sheep, or mice. These spirochetes were small, fine, delicate, and of various lengths in Giemsa preparations. They did not show the typical coils seen in *pallidum*, but rather snake-like bizarre bent ring forms turned up at both ends, or forms curved like an oesa at the end. Virus not filtrable.

A number of important observations have been made on the organism obtained from hemorrhagic jaundice by Uhlenhuth and Fromme. Gonder and Gross[2] report it as a treponema like *Treponema pallidum*, measuring 8 to 20 $\mu$ in length, with ten to thirteen turns in the recently divided forms, twenty-three to twenty-four in the longest organisms. It has the stiff appearance of *Treponema pallidum*, as if turned on a lathe. Like the spironemata and the cristispiras, it is not a true three-dimension spirally wound organism, but is wave-like, the waves being in a single plane. This is shown best by rolling the coverslip preparations, the organism appearing first as spirals, then as straight lines. Furthermore, it seems to have a straight axis or axial rod preformed in the body. The serpentine movements show that this axial rod is flexible just as Zuelzer has shown for the spirally wound *Spirochæta stenostrepta*, which also has an axial rod. Gonder and Gross believe that *icterogenes* has terminal threads about one-third the body in length, in dark field slightly refractile, in stained preparations pale and thinner than the body, irregularly wound. These are not flagella, have no inherent motility, and do not take part in the spirals of the organism. The motility is like that of *Treponema pallidum*, bending serpentine lashing movements of the whole body, both backward and forward. In this movement the wave-like undulations of the body are retained. The lashing movements are not so strong as in the spironemata, that is, the body of the organism is more rigid. There is no rapid spring-like contraction and expansion of the whole body curve like that seen by Zuelzer in the Cristispira. The motility is due to an actively motile plasma body surrounding a straight elastic axial rod, such as Schaudinn has worked out for *Treponema pallidum*. Division is transverse only. A light area appears in the middle of the organism, which now thins out and stretches at this point till it breaks, and two daughter individuals are formed. Gonder and Gross believe that this spirochete is a treponema and call it *Treponema icterogenes*. Zuelzer,[3] working with Uhlenhuth and Fromme's spirochete, regards it as closely related to the true spirochetes, especially *Spirochæta plicatilis*. She describes the body as spirally wound with a thick middle piece and two hook-like pointed tapering ends, each with a strongly refractile end-piece or terminal body. These are the free projecting ends of the elastic axial thread. The organism is free from nucleus and nucleoproteins. It divides by transverse division and shows multiple division up to five.

Uhlenhuth and Kuhn[4] believe that Weil's disease may be transmitted by biting flies, *Stomoxys calcitrans*. Uhlenhuth[5] kept the organism alive for eight weeks in sterile tap water with moor mud added. It was destroyed at 50° to 55° C. in one-half hour, by 1 per cent. HCl in ten minutes, by normal stomach juice in thirty minutes, and in a short time by 1:1000

acetic acid, rabbit and guinea-pig bile, and the acid urine of man. It grew best in distilled water with serum added, but did not survive in salt water in which the *pseudo-icterogenes* lives.

1. Uhlenhuth and Fromme: Med. Klin., 1915, xi, 1202.
———: Ztschr. f. Immunitätsforsch., 1916, xxv, 317.
2. Gonder and Gross: Arch. f. Protistenk., 1918, xxxix, 62.
3. Zuelzer: Arb. a. d. K. Gsndhtsamte, 1918, li, 159.
4. Uhlenhuth and Kuhn: Ztschr., f. Hyg., 1917, lxxxiv, 517.
5. Uhlenhuth: Deutsche med. Wchnschr., 1917, xliii, 1553.

### LEPTOSPIRA ICTEROIDES Noguchi

Discovered by Noguchi[1] in the blood, liver, and kidneys of guinea-pigs inoculated with the blood of yellow fever patients at Guayaquil.. Found also in a few instances in the blood of patients examined by darkfield illumination and in one instance in the kidneys.[2] It was cultivated originally three times from the blood of yellow fever patients and once from the liver emulsion. A similar organism was described earlier in yellow fever in one case by Stimson[3] and given the tentative name of *Spirochæta interrogans*. Noguchi and Kligler[4] have obtained *Leptospira icteroides* from cases of yellow fever at Merida, Yucatan, and Peru, Perez Grovas[5] in cases in Mexico, Le Blanc[6] in Vera Cruz, Mexico, and Noguchi and his co-workers[7] in Northern Brazil. Definite serological distinctions have been found between *Leptospira icteroides* and *Leptospira icterohemorrhagiæ* by Noguchi[8] and by Noguchi and Kligler.

**Motility** active, vibratory, progressive, and twisting. The parasite is extremely flexuous and in cultures bores into the semisolid material. When the body passes through a semisolid substance it assumes a serpentine aspect with few undulations, the elementary spiral winding undergoing no modification. The organisms show marked flexibility. In fluid media the movements are fewer and quite characteristic. One end is usually bent in the form of a graceful hook, and the organism, while rapidly rotating, proceeds in the direction of the straight end, the hooked end apparently serving as a kind of rear propeller. The rapid movement of the organism makes it appear like a chain of minute dots.

**Morphology.**—*Leptospira icteroides* resembles *Leptospira icterohemorrhagiæ* and *Leptospira hebdomadis* in morphology, but is slightly smaller. It measures 4 to 9 $\mu$ in length and 0.2 $\mu$ in width. Its extremities are tapering, ending in immeasurably thin points (Noguchi[8]). The curves are short and frequent, forming a zigzag line at angles of about 90 degrees. Each section measures about 0.25 $\mu$. The filament has the appearance of being minutely wound. One or both ends are curved or bent on the organism, forming peculiar hooks. Division transverse.

**Staining Reactions.**—*Leptospira icteroides* cannot be stained by ordinary anilin dyes. It can be stained with Giemsa or other polychrome stains, Wright or Leishman, after fixation with osmic acid. When stained with Fontana or carbol gentian-violet after a mordant of 5 per cent. tannin + 1 per cent. phenol, the organism appears as a moderately heavy slightly undulated filament without a clear elementary indentation. Peculiar forms representing a letter C or S are found. Specimens fixed with methyl alcohol seldom retain the elementary spirals.

**Cultivation.**—Cultivated first by Noguchi from patients by the use of 1 part of non-immune blood to 3 parts of Ringer's solution, and in the same mixture rendered semisolid by the addition of small quantities of melted neutral 0.3 per cent. agar. About 0.5 to 1 c.c. of the patient's citrated blood was added to the semisolid agar cooled to 42° C. The warm Ringer's solution was then poured on the semisolid portion of the agar and another ½ to 1 c.c. of blood added. The culture was covered with paraffin oil. The plasma in the patient's blood forms loose fibrin in the upper part of the culture tubes. In subsequent cultures 0.5 to 1 c.c. human or rabbit's citrate plasma was added to the upper portion to give a loose cobweb fibrin. This favors the growth of certain strains. The organism was recovered from guinea-pigs by the same method, blood being taken from the heart just before death and the citrate plasma used. Cultivated also on a similar medium made from fresh rabbit serum 100 parts, distilled water 800 parts, and 2 per cent. nutrient agar (*p*H 7.5) 100 parts. By this method the necessary hemoglobin is obtained from the patient's blood.

The slightest bacterial contamination prevents the cultivation of *Leptospira icteroides*. Growth occurs only when free oxygen has access to the cultures. Oxygen supply should not

be excessive and is best obtained by a thin layer of paraffin oil poured over the surface. A certain amount of blood-serum, not over 10 per cent., is essential for growth. Substances like peptone, meat extract, and carbohydrates do not favor or impede growth. Optimum growth in media with a reaction slightly alkaline to litmus, not stronger than 0.025 N. Good growth in neutral, not in acid, media. Phenol red in media is gradually turned pinkish. Growth more rapid at 37° C. than at 25° to 26° C., but organisms remain viable longer at the lower temperature. No growth above 42° C. or below 10° C. No change produced in red blood-corpuscles or in the hemoglobin. The serum in the media remains transparent. No visible change occurs in culture media except for a grayish haze which develops over the surface of the solid or semisolid portion in richly growing old cultures. The organisms entangle themselves in the meshes of the semisolid agar or the loose fibrin.

**Pathogenic Action.**—The blood of patients with yellow fever produces typical lesions in guinea-pigs (Noguchi[9]). The animals show a rise in temperature after three to six days from intraperitoneal or subcutaneous inoculation, a hyperleukocytosis followed by a leukopenia, with albumin and casts in the urine which is diminished in volume. The fever lasts a few days, then the temperature becomes subnormal and jaundice develops. At the same time hemorrhages occur from the gingival and nasal mucosa and from the anus. At autopsy there is a deep jaundice of the skin and sclera, the liver is fatty and yellow, the kidneys swollen and hyperemic, often showing hemorrhages, and hemorrhagic areas occur in the lungs and in the gastro-intestinal mucosa. *Leptospira icteroides* is present in the blood, liver, and kidneys of the infected animals, but not in the urine. Similar lesions may be produced in normal guinea-pigs by the isolated organism.

Infection also occurs in guinea-pigs after subcutaneous or percutaneous inoculation and administration by mouth (Noguchi[10]). Here the incubation period is twenty-four hours longer.

In experimental guinea-pig infections the blood becomes infectious forty-eight hours after inoculation and remains so for six to seven days. The organism disappears before death. They increase in the liver or kidney, but disappear as the jaundice and other symptoms become exaggerated. When death occurs leptospiras are rare in the blood and organs and it is difficult to obtain positive cultures. Rarely cultures may be obtained from moribund animals even when leptospiras cannot be demonstrated by the dark field.

The blood from yellow fever cases and cultures of the organisms have no action on ring-tail monkeys, rabbits, cats, guatuses, weasles, sloths, pigeons, ground doves, blue birds, mantas, blackbirds, parrakeets, reedbirds, toucans, blancos, donkeys, horses, sheep, or pigs.

Infection can be caused both by the blood and organs of yellow fever patients and by cultures, in very young dogs not more than six to seven weeks old, and in marmosets by the same methods of inoculation. The incubation period shows the same variations. The hemorrhagic diathesis is most pronounced in guinea-pigs, less in marmosets, least in dogs. The febrile reaction is the same in guinea-pigs and marmosets. Dogs show less febrile reaction, less fever, less albumin, fewer casts in the urine, and less jaundice. They have black and bilious vomit during life. At autopsy dogs have practically no subcutaneous hemorrhages and only a few minute ecchymoses in the lungs.

*Leptospira icteroides* is also virulent to the Brazilian monkey, *Cebus macrocephalus*.

The lesions in the experimental disease in guinea-pigs are like those seen in yellow fever in man. Perrin[11] reports wide-spread fatty degeneration in the liver, which affects the entire protoplasm and nucleus of the hepatic cell. Wandstrom[12] has observed *Leptospira icteroides* in all the tissues of guinea-pigs, especially when hemorrhages were present. They were most numerous in the liver and kidney. He did not find the wide-spread fatty degeneration of those organs reported by Noguchi and Perrin.

The virulence is best established on guinea-pigs, 300 to 350 grams in weight, by intraperitoneal inoculation of cultures two to three weeks old. These contain 50 to 100 leptospiras to each field. The minimum lethal dose varies from 0.00001 to 0.001 c.c. Some animals resist larger quantities and some have almost a complete natural immunity. Artificial cultures gradually lose virulence.

**Resistance.**—*Leptospira icteroides* is destroyed by the slightest bacterial contamination. Destroyed by heating to 55° C. ten minutes, by freezing and thawing, by complete desiccation. It is destroyed more rapidly in the presence of bacteria like *Bacillus coli*, *Bacillus lactis aërogenes*, *Bacillus subtilis*, *Bacillus mesentericus*, *Bacillus pyocyaneus*, the pneumococcus, and hemolytic streptococci. The leptospiras may live, however, with non-putrefactive or non-acid-producing bacteria. Killed in five minutes by 2 per cent. phenol or 0.1 per cent. bichlorid of mercury. They die out in a short time (twelve hours) in a piece of infected liver or kidney kept at 10° C. Dissolved in 10 per cent. sodium taurocholate, sodium glycocholate, or sodium cholate. Not injured by saponin. Dissolved in human or animal bile in concentrations of over 10 per cent.

**Filtration.**—*Leptospira icteroides* can be filtered from an emulsion of the guinea-pig or kidney through a Berkefeld V and N. under suction. The bacteriologically sterile filtrates kill guinea-pigs in 10 c.c. doses in seven and one-half to eight days, the leptospiras being present in the blood of the animal before death. Noguchi suggests that this infection may

be due to granules, since cultures containing granules but no spirochetes produce infection in guinea-pigs, the typical organisms appearing in the animal. This phenomenon is like the granular phase described by Balfour[13] for Sudanese fowl spirochetosis.

**Transmission.**—According to Noguchi[14] symptoms and lesions closely resembling yellow fever in man may be induced in guinea-pigs by the bite of the female *Stegomyia calopus* (*fasciata*), which has previously sucked the blood of yellow fever patients or animals experimentally infected with *Leptospira icteroides*. Twelve days are necessary before the mosquito becomes infectious after biting man, eight days after feeding on guinea-pigs. Probably more leptospiras are present in the blood of guinea-pigs than in man. The percentage of guinea-pigs which become infectious is very small. This may be explained by the fact that the female *Stegomya* takes up 0.01 c.c. of blood or less, while 0.1 to 2 c.c. of blood from a man at the height of the disease are necessary to produce infection. The leptospiras survive in the aseptic body cavity of the *Stegomyia*. Since *Leptospira icteroides* can penetrate the intact skin or pass through a bacteriological filter and multiplies steadily at a temperature of 18° to 37° C. (the climate of tropical countries) the mosquito transmission of *Leptospira icteroides* is in conformity with the mosquito transmission of the virus of yellow fever.

**Immunity Reactions.**—Following an attack of the artificial disease guinea-pigs become immune to subsequent infection. Many animals show only a temporary febrile reaction and slight jaundice, with a rapid recovery. These animals are subsequently immune. The blood shows a positive Pfeiffer phenomenon (Noguchi[15]). The blood of individuals who have recovered from yellow fever gives a positive Pfeiffer reaction with *Leptospira icteroides* either in culture or in an organ emulsion. Agglutinins and complement-fixing bodies are likewise present in the blood of infected animals and man (Noguchi[16]). Horses can be immunized with *Leptospira icteroides* and produce a powerful immune serum, 1 c.c. neutralizing 5,000,000 minimum lethal doses (Noguchi[17]). In guinea-pigs a polyvalent immune serum of high potency protects the animal against the progress of the infection when given in the early stages.

1. Noguchi: J. Exper. M., 1919, xxix, 547.
2. Noguchi: J. Exper. M, 1919, xxx, 87.
3. Stimson: Pub. Health Rep. U. S. Mar. Hosp. Serv., 1907, xxii, 541.
4. Noguchi and Kligler: J. Exper. M., 1921, xxxii, 601, 627.
5. Perez Grovas: J. Am. M. Ass., 1921, lxxvi, 362.
6. Le Blanc: See Noguchi and Kligler, J. Exper. M., 1920, xxxii, 601.
7. Noguchi et al: Experimental Studies of Yellow Fever, Monogr. of Rockefeller Inst. Med. Research, August, 9, 1924, No. 20. *Also:* J. Am. M. Ass., 1924, lxxxiii, 820.
8. Noguchi: J. Exper. M., 1919, xxx, 13.
9. Noguchi: J. Exper. M., 1919, xxix, 565.
10. Noguchi: J. Exper. M., 1919, xxix, 585.
11. Perrin: Am. J. Trop. M., 1923, iii, 27.
12. Wandstrom: J. Infect. Dis., 1924, xxxiv, 110.
13. Balfour: Internat. Cong. Med., 1914, xxi, 471.
14. Noguchi: J. Exper. M., 1919, xxx, 401.
15. Noguchi: J. Exper. M., 1919, xxx, 9.
16. Noguchi: J. Exper. M., 1920, xxxi, 135.
17. Noguchi: Lancet, 1922, i, 1185.

### LEPTOSPIRA HEBDOMADIS (Ido, Ito, and Wani) Noguchi

Named first *Spirochæta hebdomadis* by Ido, Ito, and Wani.[1] Called a leptospira by Noguchi.[2] The causative agent in "seven-day fever" in Fukuoks, Japan, the disease, however, being somewhat widely distributed in Japan. The organisms were found in the livers of guinea-pigs dying from inoculation with patient's blood on the third and fourth day of the disease. In guinea-pigs the jaundice and nephritis are mild and often lacking. Closely resembles *Spirochæta icterohemorrhagiæ*. Somewhat larger than *leptospira icteroides*. Differentiated from these species by animal experimentation and immunological tests. Found in the kidneys of wild mice, *Microtus montebelli*, which apparently carry the infection and disseminate it through the urine. Also found in the blood of patients with the disease on the second, third, and fourth day and in their urine, usually after the eighth day, and as late as the thirty-ninth day. Stains best with Giemsa.

Koshina, Shiozawa, and Kitayama[3] have recently shown that autumn fever in Japan, *Akiyami*, is also caused by leptospiras, one of which is serologically identical with *Lepto-*

*spira hebdomadis*, while the other is more virulent and serologically distinct from both *Leptospira hebdomadis* and *Leptospira icterohemorrhagiæ*. The latter is harbored in the field-mouse, *Microtus montebelli*.

1. Ido, Ito, and Wani: J. Exper. M., 1918, xxviii, 435.
   ———: J. Exper. M., 1919, xxix, 199.
2. Noguchi: J. Exper. M., 1919, xxx, 13.
3. Koshina, Shiozawa, and Kitayama: J. Exper. M., 1925, xlii, 873–895.

### Leptospira aqueductum (Uhlenhuth and Zuelzer)

Described as *Spirochæta pseudoicterogenes aqueductum* by Uhlenhuth and Zuelzer in fresh water of aqueducts. Widely distributed. Called "*aquatilis*" by Hoffmann. Not to be differentiated morphologically from *Spirochæta icterogenes*. Also thicker, more loosely wound, very small, and delicate forms occur. Ends not so clearly pointed, more half-circular. Not cultivated.

Uhlenhuth and Zuelzer: Centralbl. f. Bakt. 1. Abt., Orig., 1921, lxxxv, Ber. d. f. Ver. d. Mik., p. 141.

### Leptospira salina (Uhlenhuth and Zuelzer)

Reported as *Spirochæta pseudoicterogenes salina* by Uhlenhuth and Zuelzer from salt water. Not to be differentiated from *Spirochæta icterogenes*. Not cultivated. Survives long periods in salt water. *Pseudoicterogenes aqueductum* and *icterogenes* do not survive in salt water.

Uhlenhuth and Zuelzer: Centralbl. f. Bakt. 1. Abt., Orig., 1921, lxxxv, Ber. d. f. Ver. d. Mik., p. 141.

### Leptospiras in water

Noguchi[1] has found leptospiras in salt water and fresh water pools. They are like *Leptospira icterohemorrhagiæ*.

Short minute forms occur, measuring 0.2 by 3 to 4 $\mu$ and long heavy forms measuring 0.4 by 14 to 15 $\mu$. The spirals may be so tightly set together that the organism appears like a series of flat disks. There are probably two different species, neither of which is the leptospira of hemorrhagic jaundice. Impure cultures were obtained on leptospira media. Noguchi's organisms were not pathogenic to guinea-pigs and rats. Zuelzer,[2] however, reports free-living spirochetes (leptospiras) which produce disease in guinea-pigs. An immune serum prepared from them was active against *Spirochæta icterogenes* (*Leptospira icterohemorrhagiæ*).

1. Noguchi: N. York State J. M., 1922, xxii, 426.
2. Zuelzer: Centralbl. f. Bakteriol. [etc.], 1922, lxxxix, 171.

### Leptospira dentium Hoffmann

Named first *Spirochæta trimerodonta* by Hoffmann,[1] later *Leptospira dentium*.[2]

Stained best by Benian's Congo-red (1 drop 2 per cent. aqueous Congo-red followed by 1 per cent. HCl in alcohol, Hoffmann[3]). Also stains by Loeffler's flagella stain and China blue. The organisms show terminal bodies with thin threads which have no spirals, ring forms, and sling forms. They resist 10 per cent. saponin, retaining their motility, which is also retained seventy-eight hours in dark-field preparations in room temperature. After twenty-four hours at 37° C. all the leptospiras are motionless.

Found in 40 per cent. of the normal individuals examined in Bonn.

1. Hoffmann: Deutsche med. Wchnschr., 1920, xlvi, 257.
2. Hoffmann: Deutsche med. Wchnschr., 1920, xlvi, 625.
3. Hoffmann: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1921, lxxxvi, Orig., 133.

### Leptospira bovis Noguchi

Found by Noguchi in gastric mucosa of the ox.

Noguchi: N. York State J. M., 1922, xxii, 426.

### Miscellaneous spiral organisms

*Spirochæta elusa* Wolbach and Binger.
*Spirochæta morsus muris* Futaki, Takaki, Taniguchi, and Osuma.
Spirochetes in malignant tumors.
Spirochetes in swine.
*Spironema Noguchii* Strong.

### Spirochæta elusa Wolbach and Binger

A free-living filtrable organism isolated from the water collected from the shores of Jamaica Pond, Boston, by Wolbach and Binger.

**Morphology.**—Small actively motile organisms varying in size from short forms, 4 to 8 $\mu$ in length with one or two spiral turns, to long motionless filamentous forms. The morphology varies with the medium and the presence of oxygen. The long filamentous forms occur only in hay infusions. The distance from crest to crest of the spirals is about 1.5 to 2 $\mu$, the amplitude (thickness ?) $\frac{1}{2}$ to 1 $\mu$. In cross-section the body of the organism is round, not flat. The ends taper slightly to rather blunt terminations. Delicate terminal flagella can be seen with dark field illumination, one or more, often on both ends. The protoplasm always shows small granules. Coiled cyst-like forms are abundant in neutral 1 per cent. dextrose hay infusion kept under anaërobic conditions at 30° C. and under aërobic conditions at 37° C. With dark field illumination they appear as slightly refractile vesicles containing the body of the spirochete. The organisms multiply by transverse division, nothing suggestive of longitudinal division having been seen.

**Filtrability.**—The short forms pass through the pores of a Berkefeld V filter which holds back cultures of *Bacillus prodigiosus*, micrococci, and certain tiny bacilli. They also pass through a Berkefeld N filter and a Berkefeld W. Positive pressure, 16 to 20 pounds to the square inch, suction from a water aspirator and also gravity suffice to force the organism through the filters. Attempts to filter through a Chamberland F and Chamberland B were negative. The organism apparently passes through the filters in its spiral form, although proof was not presented that another filtrable stage does not exist.

**Motility.**—The spirochetes were actively motile when first isolated. This property was lost after several generations and restored by filtration. The short forms have a rapid darting movement like that of motile bacteria, while the long forms move only sluggishly. In agar jelly the organisms rotate about their longitudinal axis and exhibit corkscrew or boring-like motions. The bodies are flexible and the long forms show sinuous movements.

**Staining Reactions.**—Stained best in dried films fixed in alcohol and treated with dilute carbolfuchsin or Giemsa. The protoplasm is granular. The body stains bluish-red with Giemsa, the granules pure red. By osmic acid fixation and decolorization followed by Giemsa the protoplasm is bluish and the granules red. The flagella can be stained with the Muir-Pitfield stain and by the silver method of Stevens. They are extremely delicate, 3 to 5 $\mu$ long, and look like the flagella of bacteria. They are apparently single and polar. In old cultures the terminals are swollen to a diameter several times that of the spirochete and wavy forms show strings of granules connected by faintly staining bands. The organisms often show lateral buds as well as terminal enlargements, staining red by Giemsa.

**Methods of Cultivation.**—*Spirochæta elusa* grows in hay infusion made up as follows: One hundred and fifty grams of hay is finely cut and soaked in 1 liter of water half an hour. The water is decanted and 1 per cent. dextrose added. The reaction is adjusted neutral to phenolphthalein. It is then sterilized by filtration through a Chamberland F filter. In this medium the organisms produce a heavy turbidity in forty-eight hours. In old hay infusion cultures the turbidity is heavier with a fairly heavy grayish-white sediment. The cultures show slight iridescence. The spirochete also grows on a solid medium made from equal parts of 3 per cent. agar jelly and dextrose hay infusion. The filtered hay infusion may be mixed with the melted agar cooled to 50° C., or the mixture may be sterilized in the autoclave. The young surface colonies are transparent and look like droplets of moisture. Older surface colonies are raised, disk-like, circular, with entire edges and slight central nipple-like elevations. They are smooth, moist, slightly iridescent, translucent, grayish-white, soft, and slightly viscous. Stab cultures show a beaded growth along the line of puncture, $\frac{1}{2}$ to 1 cm. in depth, made up of small colonies.

Deep colonies are spherical and whitish with filamentous projections from the periphery into the medium.

In fermentation tubes of dextrose hay infusion there is a slight growth in the closed arm.

Optimum temperature 30° C. Growth also takes place at 20° to 23° C. and poorly at 37° to 38° C.

No growth on ordinary laboratory media or in Cohn's synthetic medium. Very slight growth on potato and in Uschinsky's medium.

Destroyed at 45° C. in five minutes and on drying on cotton cloth three days. Non-pathogenic.

**Remarks.**—This organism was described tentatively by Wolbach and Binger as a spirochete. They recognized its resemblance to the spirilla as defined by Migula, but regarded it as more closely related to the treponemata than to any other group. The presence of flagella would place it among the spirilla.

Wolbach and Binger: J. Med. Research, 1914, xxv, 9.

### SPIROCHÆTA MORSUS MURIS Futaki, Takaki, Taniguchi, and Osumi

Described originally as a spirochete by Futaki, Takaki, Taniguchi, and Osumi[1] in rat-bite fever and obtained by them by the inoculation of skin tissue and blood of a patient into monkeys, guinea-pigs, and white rats. All the animals became infected and the disease was transmitted to other animals. The organism was found microscopically in the pieces of excised skin of the patients and in the swollen lymph-glands. The organism was named *Spirochæta morsus muris*. It should probably be classed with the spirilla because of its morphology. Subsequently Ishiwara, Ohtawara, and Tamura,[2] by exposing guinea-pigs to the bite of rats, *Mus decumanus*, produced disease in 10 out of 80 rats, and found organisms like those of Futaki. Disease was also produced in a *Pithecus rhesus*. Kaneko and Okuda,[3] in an autopsy on an individual who had died of rat-bite fever, found spirochetes (spirilla) like Futaki's *Spirochæta morsus muris*.

**Morphology.**—Spiral organism comparatively short and thick in stained preparation of the blood of the patient and of inoculated animals. It measures 1.5 to 2 $\mu$ in thickness and 5 to 6 $\mu$ in length. Including its flagellum it measures 6 to 10 $\mu$. Swollen bodies appear in the blood and in lymph-nodes. In cultures larger organisms are found, up to 12 to 19 $\mu$ in length and also minute forms 1.5 to 2 $\mu$ long. Number of curves five to six, occasionally more, ten to nineteen. The curves are regular and sharp, usually one curve per micron, in the organisms from lymph-nodes. In cultures the angle of the curve is obtuse and some spirochetes show one curve in a length of 2 $\mu$. Rarely, especially when degeneration sets in, the curves become irregular. No undulating membrane. Ends pointed and pass into slender flagella, usually from one end, occasionally from both ends. Flagella usually single, may be double or multiple. They have approximately the same thickness and measure 2 to 3 $\mu$. Division transverse. Degenerate forms common in cultures. Dissolved in 0.1 per cent. sodium taurocholate and saponin (0.1 per cent.).

**Motility** in the blood, like that of the vibrios. The organisms move rapidly across the field of vision. In agar dissolved and cooled to 45° C. and mixed with fresh blood the movements are spasmodic, the spirals passing freely backward and forward. The short organisms have a vibrio-like motion. The long organisms show flexion and rotation, stretching and darting movements, the flagella whipping and winding movements. Cultivated organisms with long bodies are inactive, showing only a slight wriggling. In animal blood at 22° to 37° C. the motility ceases in twenty-four to forty-eight hours.

**Stains** easily by Giemsa, Loeffler's methylene-blue, Ziehl's solution and aqueous gentian-violet. Gram-negative. Silver impregnation easily accomplished. With Giemsa the bodies are deep violet-red. The flagella may or may not be revealed by Giemsa or by silver impregnation, but are shown by India ink. In the tissues the best results are obtained by Levaditi's silver method. The bodies of the organisms are spindle-like. Flagella appear yellowish-brown by this method. In cultures, especially in Shimanine's medium, one or more chromatin bodies appear by Giemsa's stain. Adachi[4] finds that the flagella are clearly visible by dark field illumination and may be stained by alkalinized Akashi solution, a modification of Giemsa. Films best fixed with vapor of osmic acid. Number of flagella varies, sometimes one large, sometimes several small ones. In the living condition they are spiral. Long forms may have flagella rising in the middle of the body.

**Cultivated** successfully in Shimanine's medium. Growth is noted after fourteen days at 37° C. No liquefaction or other changes occur in the medium. No odor.

**Virulence.**—When human material is inoculated into mice the organisms appear in the blood in seven to thirty days. Mice inoculated from infected mice show the spirochetes in four days. Mice survive and retain the spirochetes several months. House rats and white rats also develop spirochetosis (spirillosis). Guinea-pigs and monkeys seldom do so. Virulence of organisms increased by passage through mice and rats. Death rare in inoculated animals. At autopsy swelling of the spleen, hyperemia, congestion and swelling of the liver, hemorrhages of kidney and lung are often observed. The type of fever in monkeys is not like rat-bite fever observed in man.

*Shimanine's Medium.*—Sodium nucleate 0.5 to 0.75 gram.
Horse serum, 100 c.c.
Shake till sodium nucleate is dissolved.
Pass $CO_2$ through medium till it becomes transparent.
Heat to 60° C. one-half hour on three successive days. On fourth day heat to 65° C. for thirty minutes. Medium now separates into fluid and solid coagulated portion.

**Distribution.**—Kaneko and Okuda report that Futaki's organism has been found once in rats in nature, one *Mus rattus alexandrinus* (roof rat) producing spirillosis by the transmission of its blood to white mice. The strain of spirochte (spirillum) was like that found in man. Organisms have not been found in the saliva or mouths of house rats or other animals in whose blood it exists. Kaneko and Okuda produced spirochetosis (spirillosis) by allowing house rats to bite guinea-pigs, in one out of fifteen instances. They suggest that the organisms found in *Mus decumanus* by Carter[5] and by Mezinescu[6] may be the same as the forms found in rat-bite fever. The source of infection in man is not clear, since organisms are not present in the saliva of the infected animals. They may come from the blood by hemorrhage into the saliva. Salvarsan has a marked effect on mice infected with the organism. This is in conformity with the observation of Taniguchi and Hata[7] that salvarsan has a definite effect on rat-bite fever in man. In rat-bite fever the spirochetes (spirilla) disappear from the blood of the patient on recovery and reappear during relapse. Further surveys in Japan indicate that about 3 per cent. of house rats harbor Futaki's organism. Crohn[8] reports that 54 cases of rat-bite fever have been recorded in America. Shattuck and Theiler[9] have recently described another instance and Mooser[10] has noted 2 cases in Mexico City.

**Immunity.**—Ido, Ito, Wani, and Okuda[11] state that the serum of patients who have recovered from rat-bite fever contains immune bodies which destroy the spirochetes (spirilla). In the convalescent period the immune bodies are not so powerful as those found in hemorrhagic jaundice (from *Leptospira icterohemorrhagiæ*).

1. Futaki, Takaki, Taniguchi, and Osumi: J. Exper. M., 1916, xxiii, 249. *Ibid.*, 1917, xxv, 33.
2. Ishiwara, Ohtawara, and Tamura: J. Exper. M., 1917, xxv, 45.
3. Kaneko and Okuda: J. Exper. M., 1917, xxvi, 363.
4. Adachi: J. Exper. M., 1921, xxxiii, 647.
5. Carter: Scient. Mem. Med. Army of India, 1887, iii, 45.
6. Mezinescu: Compt. rend. Soc. de biol., 1909, xliv, 58.
7. Taniguchi and Hata: München. med. Wchnschr., 1912, xliv, 854.
8. Crohn: Arch. Int. Med., 1915, xv, 1014.
9. Shattuck and Theiler: Am. J. Trop. M., 1924, iv, 453.
10. Mooser: J. Exper. M., 1924, xxxix, 589.
11. Ido, Ito, Wani, and Okuda: J. Exper. M., 1917, xxvi, 377.

## SPIROCHETES IN MALIGNANT TUMORS

A number of spirochetes have been noted in malignant tumors, but no evidence exists of any causal relation to the neoplasms. None of them have been cultivated. The most important are *Spirochæta microgyrata* described by Lowenthal,[1] *Spirochæta Lowenthali* described by Lowenthal and named by Besson,[2] *Spironema microgyrata* var. *Gaylordi* described by Gaylord[3] as a spirochete and named by Calkins,[4] *Spirochæta gangrenæ carcinomatosæ*, found by Kreinitz[5] and named by Hoffmann,[6] and *Spirochæta pseudopallida* of Mulzer.[7] Spirochetes in tumors have also been described by Borrel,[8] Hoffmann, Ewing,[9] and Beebe.

A spirochete has been described in vaccinia by Bonhoff[10] as *Spirochæta vaccinæ*. Blanchard[11] has recorded as *Spirochæta pyogenes* the organism

found by Mezinescu in tuberculous pyelitis. *Spirochæta lymphatica* has been found in acute lymphatic leukemia and in Hodgkin's disease by Proescher and White.[12]

1. Lowenthal: Berl. klin. Wchnschr., 1906, xliii, 283.
2. Besson: p. 736.
3. Gaylord: Seventh Ann. Rep. New York State Cancer Lab., 1918.
4. Calkins: J. Infect. Dis., 1907, iv, 171.
5. Kreinitz: Deutsche med. Wchnschr., 1906, xxxii, 872.
6. Hoffmann: Berl. klin. Wchnschr., 1905, xlii, 880.
———: Deutsche med. Wchnschr., 1906, xxxii, 967.
7. Mulzer: Berl. klin. Wchnschr., 1905, xlii, 1144.
8. Borrel: Compt. rend. Soc. de biol., 1905, lviii, 770.
9. Ewing: N. York State J. M., 1907, vii, 177.
10. Bonhoff: Berl. klin. Wchnschr., 1905, xlii, 1142.
11. Blanchard: Semaine méd., 1906, xxvi, 1.
12. Proescher and White: München. med. Wchnschr., 1907, liv, 1868.
———: J. Am. M. Ass., 1907, xli, 1115.

### Spirochetes in Swine

Spirochetes were originally noted by Smith[1] in small ulcers of the large intestines of hogs. They were fine spiral organisms with 2 to 3 waves. In preparations stained by methylene-blue the wave length was about 2 $\mu$. Dodd[2] has observed long very slender spirochetes in cutaneous lesions in pigs, not in the blood-stream. They were 9 to 26 $\mu$ long, average length 14 to 16 $\mu$. Both ends pointed. They occurred as spirals, semicircles, and long straight threads. Spiral turns varied from 2 to 6. Organisms arranged singly, in twos, threes, and in clusters of 20 or more. Stained by ordinary dyes, not by Gram or Claudius. Stains well by Romanowsky or its modifications, especially Giemsa. Unstained spots or vacuoles appear in the organism. No nucleus. Not cultivated. Not transmitted to other animals. Cleland[3] has recorded spirochetes in the large oval fibrous tumors with seropurulent contents, which develop at the site of castration in pigs. They stain with weak carbolfuchsin or Leishman's stain and are found along with cocci and bacilli. They vary in length from 6 to 12 $\mu$ thickness, very fine, running up to thickness of the tubercle bacillus. Spirals 3 to 4 in number, quite regular or irregular, forming a right angle. These organisms resemble the spirochetes described by Dodd.

1. Smith: Centralbl. f. Bakteriol. [etc.], 1904, xvi, 324.
2. Dodd: J. Comp. Path. and Therap., 1906, xix, 216.
3. Cleland: Parasitology, 1908, i, 218.

### Spironema Noguchii Strong

Found by Strong in dermal spirochetosis in the tropics. Measures 14 to 30 $\mu$ in length and thicker than *Treponema pallidum* or *Treponema pertenue*. Deeply stained granules occur on the ends. Demonstrated by hardening sections in absolute methyl alcohol and staining in Giemsa.

Strong: Tr. Ass. Am. Physicians, 1925, xl, 427.

# PART VI

## INFECTIOUS MICRO-ORGANISMS OF UNDETERMINED CHARACTER

In addition to the infectious diseases of man and animals in which the causative organisms are now with greater or less certainty shown to be either bacteria or protozoa, there are a number of diseases in which the etiological agents have not been definitely cultivated outside the body or whose nature has not thus far been determined. This group includes the acute exanthemata of man, variola, varicella, and measles; certain diseases like dengue fever, papataci fever (three-day fever), typhus fever, Rocky Mountain spotted fever, and trench fever; diseases of cattle, like cattle plague (Rinderpest) and pleuropneumonia (peripneumonia, Lungenseuche); affections of horses, like horse distemper and horse sickness; and a number in other species.

All the diseases in this group may be regarded as *infectious*. They are characterized by the usual phenomena associated with the infectious diseases, such as (1) definite period of incubation; (2) fever and constitutional symptoms; (3) termination after a fairly uniform course in death or recovery; (4) characteristic pathological lesions at autopsy; (5) immunity after recovery.

In addition, the diseases are transmissible from individual to individual of the same species and frequently to other species. In some instances the infectious material, for which we may use the general term "virus," is in the secretions or excretions of the body, and the infection travels by direct contact or by the contamination of objects in the environment. This is true of variola in man and of pleuropneumonia in cattle. In such diseases infection of susceptible animals may be produced by direct inoculation with this material. In other instances the virus is in the blood of the infected; infection may be produced in sensitive individuals by direct introduction of the blood or blood-serum or may be carried by insects of various types, the so-called vectors. Whether the virus actually multiplies in the body of the vector, or is simply retained there for a certain time during which the insects are capable of producing infection under suitable conditions, has not thus far been determined with certainty.

Finally, there are several diseases in which transmission to other individuals by the direct inoculation of secretions, excretions, or blood has not yet been accomplished; for the sake of completeness these diseases may also be included in this section.

**Filtration.**—In many of the diseases considered in this group the virus in the secretions, excretions, blood, or blood-serum may be filtered through filters like Berkefeld or Chamberland bougies which do not permit the passage of ordinary bacteria. In consequence the etiological agents are frequently spoken of as *filtrable viruses*. Such a designation has obvious disadvantages. Filtration itself is a physical phenomenon in which the permeability of the filter for micro-organisms depends upon a number of factors, of which the most important are: (1) the material of which the filter is made, whether Kieselguhr, as in the Berkefeld filter, or unglazed porcelain, as in the Chamberland filters; (2) the filter mass which by adsorption and molecular attraction can take part in the process, and (3) the spaces and capillaries in the filter material. In addition, certain characteristics of the

material to be filtered are of determining importance, such as the form and elasticity of the elements, the viscosity of their surfaces, the character of the surrounding medium and its content in protein or colloidal dissolved material. Recent investigations of the intricate phenomena of filtration, especially by Esmarck, Hofstädter, Rosenthal, and Schmidt, have shown that the thin mass of the filter contains many irregularly limited hollow spaces, with a lumen from 2 to 100 $\mu$ in diameter, and fine canals communicating with these hollow spaces. Retention of particulate matter takes place in these fine canals and spaces. If filters be used for filtering Gram-positive bacteria, then sectioned and stained by Gram, the organisms are found not in aggregates, but in various areas of the compact substance which give an approximate idea of the fineness and disposition of the capillary anastomoses. The filters themselves do not offer an *absolute impermeability* to the passage of organisms. Minute bacteria, like *Spirillum parvum* of Esmarck, can be filtered. Rosenthal thinks that they often grow around the impermeable stenoses. Rosenthal also believes that the positive or negative pressure used drives the fluid with its suspended particles against the various spaces and channels and makes them go the shortest way through the filter mass. There is always some place which offers resistance and is stopped up by the organisms or particulate matter. Rosenthal regards the width of this space as the "determining pore diameter" (wirksame Porengrösse), and concludes that the passing of the organism depends partly on this determining pore diameter and the dimension of the organism. He finds the pore size for bacteria-tight filters to be 0.5 to 2 $\mu$. Schmidt estimates it for Berkefeld filters at 0.2 to 0.8 $\mu$. In both instances the size of the pore spaces is much greater than that of many small bacteria. Filtration thus can have practical value and be of distinguishing importance only when different investigators employ the same type of filter, the same pressure, same period of filtration, etc.

As far as any characterization of the objects filtered is concerned, long ago Borrel (1903) indicated that passage through a filter did not necessarily mean an invisible virus, and this has been abundantly confirmed by more recent investigation. It has been shown by Smith and Wherry that the minute bacillus of guinea-pig pneumonia passes Berkefeld V. It is now known that *Spirillum parvum* of Esmarck and *Micrococcus mesnili* of Borrel can pass through filters which were previously thought to hold back all organisms of the nature of bacteria. Wolbach and Binger found that *Leptospira biflexa* will pass Berkefeld V filters; *Spirochæta elusa*, Berkefeld V, N, and W; Noguchi finds that *Leptospira icteroides* can be filtered through Berkefeld V and N. Furthermore, the medium in which the organisms are bathed is of paramount importance. Thus in trench fever the virus in the blood is not filtrable, while in the urine it is. As Doerr has expressed it, the division of infectious agents into filtrable and non-filtrable can have no claim to scientific meaning. For this reason we prefer not to employ the term "filtrable virus," but to speak of the organisms causing these diseases as infectious micro-organisms of undetermined character.

**Type of Micro-organism.**—Many attempts have been made to discover micro-organisms in these infections, yet our information in regard to the etiological agents is still very meager. Careful histological study of the lesions and careful microscopic examination of the infectious material have revealed two main types of structures—*cell inclusions* and *minute intracellular* or *extracellular bodies*. Cell inclusions have been observed with greatest certainty in molluscum contagiosum (molluscum bodies), in variola and vaccinia (Guarnieri bodies), in rabies (Negri bodies), and in fowl pest

(Keene-Schiffmann's bodies). These inclusions are usually in cells of the ectoderm, but may be in mesodermal cells. They often lie in the neighborhood of the nucleus. They may consist of keratin-like degeneration of the cytoplasm, as in the molluscum bodies; protein-lipoids, as in fowl-pox; chromatin and plastin, as in the Guarnieri bodies; or hyaline substance, like nuclear material, as in fowl pest. Similar cell inclusions have been found by Mallory in scarlatina in epithelial cells and by Cantacuzène and Bembardt in the glandular epithelium in this disease. At the present time cell inclusions are regarded not as micro-organisms, but as reaction products on the part of the cells against the disease-producing virus. This has been made more certain by the demonstration by v. Prowazek that the Guarnieri bodies in vaccine virus may be digested away by pepsin and pancreatin and the virus yet remain infective.

In addition to the cell inclusions peculiar bodies have been found in the lesions of various diseases and in the insect vectors. These bodies are minute, non-motile bacteria-like dots of protoplasm which stain with difficulty and may be demonstrated best by Loeffler's flagella stain or by Giemsa. They are thought to divide by the formation of a dumb-bell-shaped figure as in the division of the centriole in certain animal cells, and not by simple fission as in the bacteria. Two dots appear connected by a fine line which becomes drawn out till it snaps across the middle and its two halves are retracted into the body. The bodies themselves look like grains of chromatin. They are usually found in the epithelial cells, but are occasionally inside the nucleus or in the intracellular spaces. These minute bodies have been termed "Chlamydozoa" by v. Prowazek and Herzog, and "Strongyloplasmata" by Lipschütz. The more minute forms which pass through Berkefeld filters are termed "elementary corpuscles." In the body of the infected individuals these structures grow into "initial bodies" which appear usually within the cells. v. Prowazek has described a further stage between the elementary corpuscle and the initial body as the "resting body." According to Lipschütz the Strongyloplasmata are about 0.2 $\mu$ in size, but may be larger, never under 0.1 $\mu$; he regards them as smaller than any known organism. Their filtrability is explained by the fact that they never reach 0.5 $\mu$ in size (Rosenthal's effective pore diameter). Lipschütz states that the filtration to be employed for organisms of this type should last only a short period, not more than two hours, the positive or negative pressure should be 50 to 500 mm., the temperature should be constant, and the fluid filtered should be in a dilution of 1:100.

With the knowledge now on hand in regard to these minute bodies (Chlamydozoa or Strongyloplasmata) it is impossible to determine their nature. Details of structure are lacking which if present might place them in the protozoa. They resemble small pleomorphic bacteria. Until they have been cultivated on artificial media outside the body and some of their characteristics determined, it will be impossible to come to any definite conclusion in regard to them. Recently certain of these bodies have been studied with great care in connection with typhus fever, trench fever, Rocky Mountain spotted fever, and heart-water of sheep. The observations brought out are so important that they may properly be considered in a separate section.

## RICKETTSIA BODIES

In their earlier work on typhus fever Ricketts and Wilder described minute organisms in preparations of blood stained by Giemsa which reminded them of members of the hemorrhagic septicemia group. They were estimated at about 2 $\mu$ in length and about one-third of this in breadth

(0.6 to 0.7 $\mu$), with an unstained central area or finely stained bar. These observers also noted other structures consisting of stained granules connected by an intermediate substance either stained blue or not at all. Some of the granules were deep purple, others faint blue. Ricketts and Wilder's observations on these peculiar bacteria-like bodies in typhus have not been confirmed except by Gavino and Gerard, who found a few bacillary forms in the blood, 2 by 0.5 $\mu$ in dimensions, and by Sergent, Foley, and Vialette, who have described bipolar organisms, 1 to 3 $\mu$ in length and 0.5 to 0.8 $\mu$ wide. In 1916 da Rocha-Lima reported observations made with v. Prowazek before the latter's death in which they found great numbers of minute organisms in typhus-infected lice demonstrable only by Giemsa's stain. The organisms had less sharply defined contours than bacteria and conconsisted of two substances, one staining faintly and the other, usually at the poles, taking the stain deeply. The heavily stained portions at the poles were joined by the lightly staining portion. These organisms looked like paired granules under dark field illumination. They disintegrated in water. In size the single granules were 0.3 by 0.4 $\mu$, the double granules or rods, 0.3 by 0.9 $\mu$. Da Rocha-Lima found these organisms in infected lice, but not in normal lice, in the epithelial cells of the louse's stomach. A temperature of at least 23° C. was necessary for their development in lice and a period of five days required before they could be demonstrated. Later da Rocha-Lima gave the name *Rickettsia Prowazeki* to these organisms in honor of Ricketts and v. Prowazek. Da Rocha-Lima's observations were confirmed by Töpfer and Schüssler in regard to Rickettsias in lice fed upon typhus patients. These workers failed to find the bodies in lice fed upon healthy individuals. Later Töpfer extended this work and concluded that Rickettsias are bacteria, like those described earlier by Sergent, Foley, and Vialette, the type form being a bacillus, the other forms stages in growth. Töpfer regarded *Rickettsia Prowazeki* as the cause of typhus fever. Rickettsias were also found by Otto and Dietrich in lice fed upon typhus patients and some positive agglutinations obtained with typhus blood. Finally, Rickettsia bodies were observed by Töpfer in lice in connection with trench fever and also by Jungmann and Kuczynski. Later Munk and da Rocha-Lima found Rickettsias in lice from Wolhynian fever, but also in lice fed upon cases of other diseases and upon normal individuals. These Rickettsias, however, multiplied and remained extracellular in the louse's alimentary tract. This organism was named *Rickettsia pediculi*. About the same time Nöller found Rickettsias in the louse or tick of sheep (*Melophagus ovinus*), not associated with any ovine disease. The etiological relationship of Rickettsias to disease was strongly opposed by Brumpt and by Strong, both of whom fed lice containing Rickettsias on normal individuals without the development of any ill effects.

Since these early investigations further observations of great importance have been made upon the bodies now commonly called Rickettsias. Wolbach and Todd have found them regularly in typhus fever. They describe the typhus Rickettsia as morphologically similar to bacteria, but usually smaller. The bodies occur in pairs and may be bacillary or filamentous; are non-motile; stain with difficulty by ordinary basic anilin dyes; decolorize by Gram; and are best demonstrated by the Giemsa modification of Romanowski's method. Wolbach has described similar structures in the lesions of Rocky Mountain spotted fever and in ticks which transmit the virus. He has named this organism *Dermocentroxenus Rickettsi*. It is less like bacteria than the other Rickettsias, exhibits red and blue staining material, and does not show thread-like or filamentous forms. After an intracellular stage in

the tick it floods all the tissues, diminishes in number, and leaves behind in certain tissues, including the salivary glands, forms which are different from the multiplicative forms. These Wolbach regards as resistant stages.

Sellards has cultivated a Rickettsia from animals experimentally infected with the virus of Japanese flood-fever and this has been named *Rickettsia nipponica.*

Cowdry has found a Rickettsia in the disease known as heart-water of sheep and named it Rickettsia *ruminantium.* The organisms were present both in the infected animals and in infected ticks. He regards them as the etiological agent of the disease.

Arkwright, Atkin, and Bacot have observed that bedbugs, *Cimex lectularius,* are heavily infected with Rickettsias which produce no symptoms when the bedbugs are allowed to feed on man. These have been named *Rickettsia lectularia.* They are morphologically like *Rickettsia Prowazeki,* pleomorphic, stain with difficulty, and multiply within cells.

As far as the Rickettsias in lice are concerned, these are evidently two types. One is extracellular and multiplies outside cells, in the lumen of the gut. This has been given different names, *Rickettsia pediculi, Rickettsia quintana,* and *Rickettsia Wolhynica.* The organisms stain somewhat easily, are fairly uniform, and usually somewhat oval. Wolbach and Todd regard this species as probably the cause of trench fever, but this is not entirely accepted. The other is intracellular, very pleomorphic, and multiplies exclusively within the epithelial cells of the gut of the louse. This is *Rickettsia Prowazeki* and is apparently the etiological agent in typhus fever.

All Rickettsias have insect hosts. With the pathogenic Rickettsia these insects may act as vectors and carry the disease. The organisms are transmitted to successive generations in the insect vectors, as from larva to nymph and from nymph to larva, in *Rickettsia ruminantium.*

Certain Rickettsias, like those found in typhus fever and Rocky Mountain fever, are very susceptible to heat, drying, and chemical agents. Others, like the virus of trench fever, resist when dry a temperature of 80° C. for twenty minutes, and withstand drying for many months.

Many attempts have been made to cultivate the organisms known as Rickettsias, and in a few instances these have proved successful. *Rickettsia melophagi* has been cultivated by Nöller. This organism is non-pathogenic and lives upon the cuticular surface of the stomach epithelium of the sheep louse or sheep tick, *Melophagus ovinus.* It is like the smaller bacteria in morphology and grows upon inactivated blood agar, that is, blood agar heated to 57° C. Sikora found a multiplication of *Rickettsia pediculi* when the organisms were introduced into the celomic cavity of the louse. They also grow on human blood agar at 37° C., but better on the inactivated blood agar of Nöller. They could not be cultivated on horse blood agar or rabbit blood agar. Rickettsias from the blood of typhus fever cases and from the brain and kidneys of guinea-pigs injected with typhus fever virus have been brought to cultivation by Loewe, Ritter, and Baehr. These observers used rich ascitic fluid containing a fragment of sterile rabbit or guinea-pig kidney and a small amount of 2 per cent. dextrose broth, the tubes being sealed with sterile petrolatum. Finally, Wolbach has gotten a multiplication of Rickettsias in living tissue cultures. Bacot and Segal have shown that if lice be injected with a concentrated emulsion of blood-platelets from typhus infected guinea-pigs the development of the Rickettsia can be observed in the insects. The Rickettsias are thus associated with the blood-platelets.

With our present knowledge it is unwise to come to any absolute con-

clusion in regard to Rickettsias. They are undoubtedly present in the organs and tissues of at least three diseases of man, typhus fever, trench fever, and Rocky Mountain spotted fever in animals artificially infected with the virus of another human disease, Japanese flood-fever and in animals infected with the virus of heart-water of sheep. Their constant association with the lesions offers strong presumptive evidence that they serve as etiological agents. This evidence is strengthened by their presence in the insect vectors which transmit the diseases in question. It is clear that the Rickettsias have certain characteristics, as follows:

1. They stain poorly, best by Loeffler's stain and by Giemsa.
2. They are very small, less than 0.5 $\mu$ in diameter.
3. The smaller forms may pass through filters.
4. They multiply in insects, especially in the epithelial cells.
5. They are like pleomorphic bacteria in morphology.

The following table, modified from Wolbach and Todd, gives the most important Rickettsias and their possible insect hosts. In some instances they have been actually demonstrated in these hosts.

RICKETTSIAS

| | | |
|---|---|---|
| *Pediculus humanus* (human louse) *Rickettsia Prowazeki* | | |
| | Hegler and von Prowazek | 1914 |
| | da Rocha-Lima | 1916 |
| *R. Wolhynica* | Töpfer | 1916 |
| *R. pediculi* | Muist and da Rocha-Lima | 1917 |
| *R. quintana* | Schmencke | 1917 |
| R. (Rocha-Lima) | | 1920 |
| *Melophagus ovinus* (sheep louse) *R. melophaga* | Nöller | 1917 |
| *Psocus Sp.* (dust louse). Unnamed. | Sikora | 1918 |
| *Ctenocephalus felis* (cat flea) *R. ctenocephali* | Sikora | 1918 |
| *Ctenosylla musculi* (mouse flea). Unnamed | Sikora | 1918 |
| *Dermacentor venustus* (wood tick) *Dermocentroxenus Rickettsi* | | |
| | Ricketts | 1909 |
| | Wolbach | 1918 |
| *Culex pipiens* (mosquito). Unnamed | Nöller | 1920 |
| *Leptus akamushi* (Japanese harvest mite) *R. Nipponica* | | |
| | Sikora | 1920 |
| | Sellards | 1923 |
| *Dermanogesus Sp.* (European bird mite). Unnamed | Nöller | 1920 |
| *Cimex lectularius* (bedbug) *R. lectularia* | Bacot | 1921 |
| *Trichodictes pilosus* (horse louse) *R. trichodictæ* | Hindle | |
| *Linognathus stenopsis* (goat louse) *R. linognathi* | Hindle | |
| *Lipeuris baculus* (dove louse). Unnamed | Hindle | |
| *Amblyomma hebræum* (bont tick) *R. ruminantium* | Cowdry | 1925 |

## DISEASES DUE TO INFECTIOUS AGENTS OF UNDETERMINED CHARACTER

*Man:*

Variola and vaccinia.
Alastrim.
Measles.
Mumps.
Varicella.
Dengue fever.
Pappataci fever.
Japanese flood-fever (tsutsugamushi).
Poliomyelitis.
Lethargic encephalitis.
Herpes febrilis.
Herpes zoster.

Typhus exanthematicus.
Trench fever.
Rocky Mountain fever.
Veruga peruviana.
Verruca vulgaris.
Molluscum contagiosum.
Trachoma.

*Dogs:*
Rabies (Wut).
Aujeszky's disease (Pseudowut).
Distemper (Hundestaupe).

*Cattle:*
Pleuropneumonia (peripneumonia).
Cattle plague (cattle-pest, Rinderpest).
Stomatitis papulosa bovis specifica.
Foot-and-mouth disease (aphthous fever).

*Horses:*
Infectious anemia (swamp fever).
South African horse sickness (Pferdesterbe).
Horse-pox.
Horse distemper (Pferdestaupe).
Borna's disease.

*Swine:*
Hog-cholera.

*Sheep:*
Heart-water.
Nairobi sleeping sickness.
Contagious pustulous stomatitis.
Agalactia of sheep and goats.
Sheep-pox.
Catarrhal fever of sheep (blue tongue).

*Birds:*
Fowl pest.
Fowl-pox.
Fowl diphtheria.
Leukemia of fowls.
Blackbird septicemia.
Rous' chicken sarcoma.

*Rats:*
Novy's rat disease.

*Guinea-pigs:*
Guinea-pig disease of Petrie and O'Brien.
Guinea-pig paralysis of Roemer.

*Rabbits:*
Sanarelli's myxomatous disease.
Snuffles.

*Plants:*
Mosaic disease of tobacco.

## VARIOLA AND VACCINIA

Several diseases characterized by fever, pustulent eruptions, and transmissibility to other members of the same species are described as pox or pocks. Such diseases occur in man (smallpox, variola), horses (horse-pox, sore heels), sheep (sheep-pox), cattle (cowpox), and swine (swine-pox). At

the present time it is generally believed that human pox, cowpox, and horse-pox are the same disease. Sheep-pox and swine-pox are other diseases. In man variola has existed for centuries; it was the prevalent disease in many countries during the Middle Ages. It was so wide-spread in England and Central Europe, for instance, that a large proportion of the adult population was pock-marked and all children were expected to get the disease. At that time smallpox was primarily a disease of childhood.

From early times it had been recognized that the disease is contagious, passes from person to person by direct contact, and leaves behind a permanent immunity. It was further believed that the smallpox pustules contain an infectious lymph or virus which will induce smallpox on introduction into the nose or beneath the skin of non-immunes. This led to the practice of inoculation by means of which true smallpox was produced with a lessened mortality. The disease so induced was known as *variola inoculata.* In Jenner's hands infectious material from another pox disease, that of cows, was substituted for smallpox virus. The disease following inoculation with cowpox virus was called *vaccinia* and the practice *vaccination.* The question naturally arose as to whether cowpox is an affection of cows "sui generis," or a bovine manifestation of smallpox. It was generally believed, however, that each case of cowpox in cows arose from contact with human disease, either smallpox or cowpox, chiefly by mechanical transmission of the virus from the hands of milkers. Experimental evidence in favor of the view that cowpox is modified smallpox has been obtained very slowly, despite numerous attempts to produce cowpox artificially with smallpox material. Positive results were reported as early as 1825 by Billing in Stockholm, by Brown in England, by Smith in Baltimore. Later the question was much debated, with accumulation of considerable quantities of evidence both in favor of and against this transformation. It was not until after the work of the Lyons Commission (Chauveau, Viennois, and Meynet[1]), about 1864, that Voigt[2] and Fischer[3] about 1890 showed that characteristic vaccinia was produced in cows by inoculation with smallpox material under conditions which were free from criticism. Subsequently Freyer[4] produced vaccinia in cows and obtained a lymph which was used successfully for inoculation against smallpox. The virus of smallpox was transferred also to other animals, to rabbits by Freyer, to asses by Chaumier,[5] to apes by Teissier, Duvoir, and Gastinel,[6] Stevenin, Park,[7] Brinckerhoff and Tyzzer,[8] to sheep by Voigt, to buffaloes by Gauducheau,[9] to horses possibly by the Lyons Commission. As a result of many observations it is now usually believed that human smallpox, cowpox, and horse-pox are the same disease, caused by the same etiological agent.

The first observations of importance on the infectious agent in smallpox and vaccinia are those of Pfeiffer,[10] who found peculiar bodies in the blood-serum and in the lesions of vaccinated men and animals which he regarded as protozoa, and of van der Loeff,[11] who observed similar structures in the blood of variola patients, in that of vaccinated animals and children, and in the lymph. Subsequently Guarnieri[12] succeeded in producing pathological lesions on the cornea of rabbits by inoculating it with variola material and with vaccine lymph. He observed peculiar structures both in the infectious material and in the epithelial cells of the rabbit's cornea. He observed further that the infection of the rabbit's cornea could be transmitted from animal to animal and that the same lesion was produced, characterized by the presence of the bodies inside the cells, especially in the neighborhood of the nucleus. Guarnieri named these structures *Cytoryctes variolæ-vaccinæ,* but they are usually spoken of as Guarnieri bodies.

The Guarnieri bodies are visible in unstained material, but are best brought out by hardening and sectioning the lesions and staining them with nuclear dyes. They lie in the cells of the conjunctiva, near the nucleus, but seldom inside it. They are also present in the intracellular spaces and in the connective-tissue cells of the cornea. Structurally they consist of a small mass of material staining like chromatin, surrounded by a lighter unstained halo. Occasionally one or more darker granules appear in lightly stained preparations or dumb-bell forms or spheres containing small dots of chromatin.

Guarnieri's discoveries have been abundantly confirmed, especially by Councilman[13] and by Howard and Perkins[14] in America, but there has always been some question as to the interpretation of the Guarnieri bodies. They have been regarded by some authors as the parasite itself, by others as harboring the parasite. At the present time they are usually considered to be a characteristic reaction of the cells against the virus of both variola and vaccinia. They are always present in the lesions of variola and vaccinia and are thus pathognomonic of the disease. They do not appear in the lesions of other diseases. Thus they are not present in corneal inflammation from other causes.

The smallpox virus is given off from the body through the skin and mucous membranes. It dries on clotting and permits the transmission of the disease by fomites. Loeffler[15] thinks that it may be present in the air about cases of smallpox, and persons exposed in this way contract the disease even when they have no other contact with the infectious material (aërial transmission).

Von Wasielewski[16] has kept the vaccine virus alive for 46 generations by continuous reinoculation of the cornea of rabbits. Henseval and Convent[17] have kept it alive by inoculation into the testes of rabbits, and material from such animals may be employed for the immunization of man against smallpox. The virus is destroyed at 100° C. in three minutes, at 80° C. in one hour in glycerin, at 60° C. in fifteen minutes. When dried it remains alive for months (two hundred and twenty days according to Paschen). It is rapidly destroyed by rabbit bile and sodium taurocholate. It is not injured by digestion for twenty-four hours with trypsin or pepsin. It is destroyed by acids, strong salt solutions, and ammonia.

The Guarnieri bodies cannot themselves be the virus of smallpox or vaccinia, since they do not pass Berkefeld filters. The vaccine virus has been filtered through Berkefeld and Chamberland filters by Negri[18] (1905), the variola virus by Casagrandi[19] (1908). The sheep-pox virus has been filtered by Borrel (1902). The pox materials must be rubbed up thoroughly and greatly diluted. Von Prowazek and Beaurepaire Aragão[20] find that the virus is adsorbed by colloid filters such as filters covered with 3 per cent. agar.

A number of other structures have been found in smallpox and vaccine materials. Thus Paschen,[21] von Prowazek,[22] and Fornet have observed small round bodies in large numbers in the lymph and in the specific skin lesions of vaccinia in children. These are somewhat similar to the bodies found in molluscum contagiosum, trachoma and fowl-pox, and are known as Paschen's elementary corpuscles. These corpuscles are about $\frac{1}{4}$ $\mu$ in size, round, and stain red after the use of Loeffler's mordant and carbolfuchsin. They divide by transverse fission and may show delicate threads after division. They stain blue by Giemsa, pale by thionin, carbolfuchsin, and iron hematoxylin. When unstained they appear as round, delicate, slightly refractile bodies with molecular motion, showing slight refractility under the dark field. They are destroyed by antiformin.

The Paschen corpuscles are diagnostic when studied by Paul's corneal test.[23] In this the dried variola or vaccinia secretions are rubbed up with 50 per cent. glycerin and dropped on the cornea of a rabbit which has been cocainized and then scarified by scratching with fine needles. After forty-eight hours examination with a low power lens (8 diameters) reveals small raised pointed excrescences about 1 mm. in diameter between the scratches, the rest of the cornea remaining clear. These look like small bubbles of air. The eye is now excised and dropped in sublimate alcohol. After two to five minutes milk-white turbidities appear, $\frac{1}{2}$ to 2 mm. in diameter, corresponding to the location of the virus. Microscopically the nodules consist of small areas of necrosis in which both the Guarnieri bodies and the Paschen elementary corpuscles can be found.

Paschen's corpuscles pass through Berkefeld filters and may possibly represent the virus of smallpox and vaccinia.

Scott and Simon[24] have investigated Paul's reaction from the standpoint of public health diagnosis. They have produced the reaction with material from the nose in doubtful cases of smallpox and have found the Paschen corpuscles in the lesions in the inoculated rabbit's cornea.

Recently Parker and Nye[25] have succeeded in cultivating vaccine virus in tissue cultures of cells from rabbits' testes.

A similar cultivation has been effected by Hach.[26]


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## ALASTRIM

Alastrim is a disease widely prevalent in Brazil which resembles variola except that it is rather benign. The mortality is low, but deaths do occur. It is highly contagious and leaves behind a permanent immunity to itself.

Alastrim has been studied especially by Beaurepaire Aragão[1] in Brazil and by MacCallum and Moody[2] in Jamaica. Guarnieri bodies have been reported by Carini[3] in sections from pustules and in the cornea of rabbits inoculated with the virus. According to Aragão the virus passes Berkefeld filters. It apparently passes through the placenta of women with the disease into the fetal circulation. Aragão has found Paschen corpuscles in alastrim, and this has been confirmed by MacCallum and Moody, who observed them in fresh material from alastrim pocks and in material passed through Berkefeld filters. Such filtered material apparently does not produce lesions in the cornea of rabbits. MacCallum and Moody were unable to produce lesions in rabbit's cornea with the contents of alastrim pustules.

Opinions differ as to whether vaccination with cowpox virus protects against alastrim. Many cases of alastrim have been noted among the vaccinated. The alastrim virus apparently protects against alastrim, but not against smallpox.

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## MEASLES

Measles (morbilli, rubeola) is a contagious disease of childhood characterized by an incubation period of nine days, by prodromal symptoms of sneezing and running of the eyes, which are sensitive to light, a typical rash, and a fever which disappears on the outbreak of the rash. The disease is transmissible in the prodromal stages. It is usually believed that the virus of the disease is given off during sneezing in fine particles of moisture and that a kind of infectious atmosphere is formed about the patient. Measles is highly contagious and passes rapidly to non-immunes. After one attack an immunity develops which is usually lifelong.

Measles has been transmitted to apes by Goldberger and Anderson[1] by the subcutaneous introduction of the nasal and buccal secretions taken from a case immediately before the outbreak of the rash or twenty-four to forty-eight hours after. After an incubation period of five to seven days the animals developed a disease like that in man. The virus is also in the blood in man on the first day of the eruption, in apes several days before and twenty-four hours after (Nicolle and Conseil[2]). It is not present in the blood of man or apes sixty hours after the outbreak of the exanthem. The virus may be filtered through Berkefeld filters and is destroyed in fifteen minutes at 55° C., not by freezing or drying twenty-four hours. Skin scales are not infectious.

Döhle[3] has found spindle-shaped bodies in measles blood like those found in smallpox and scarlatina.

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## MUMPS

A filtrable virus has been described in the saliva in mumps by Granata.[1] It causes a fever in rabbits lasting three days and corneal injections give rise to zoöglœa-like masses of minute bodies in the corneal cells. Wollstein[2] injected the parotid gland and the testicle of cats with filtrates from the saliva and produced a disease like mumps in man. The parotid was swollen and painful, the neighboring lymph-glands enlarged. The disease could be carried on by filtrates of the saliva of the infected animals.

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## VARICELLA

Varicella (chickenpox) is a contagious disease of man characterized by an incubation period of ten to fifteen days, fever, and a typical exanthem. It is highly contagious and passes rapidly to the non-immune. Immunity develops after an attack, usually lifelong. Second attacks may occur. Tyzzer,[1] and Keysselitz and Mayer[2] have described cell inclusions in the Malpighian layer of the skin, made up of plastin. Rivers and Tillett[3] have attempted to transmit varicella experimentally to rabbits and have encountered several viruses which cause local infiltration with leukocytes, swelling of the epithelial cells, and nuclear inclusions in the epithelial cells and in the endothelial leukocytes. These lesions resemble somewhat the lesions in varicella. These authors believe that none of these rabbit viruses is definitely related to varicella.

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## DENGUE FEVER

Dengue or break-bone fever is a mild disease which often prevails in tropical and subtropical countries, may occasionally become epidemic, and affect a large number of the population. The onset is rapid after an incubation period of one to four days. During the attack the patients have chills and fever, an inflammation of the tonsils, a peculiar reddening of the skin, headache localized in the eyes, and severe pains in the bones and joints. The knees are often affected so that the cases have a peculiar walk from which the disease is sometimes called "dandy-fever." The elevation of temperature lasts three days, then disappears rapidly with severe sweating. The sweat has a peculiar odor like that of foul straw. The acute symptoms last six to seven days and are followed by a long convalescence. The mortality is low.

Dengue fever has been transmitted to healthy men by Ashburn and Craig[1] by the use of unfiltered blood. These authors got seven positive results in eleven cases. The disease could also be transmitted by blood filtered through porcelain bougies which held back *Bacillus melitensis*.

Graham[2] first showed that dengue fever is transmitted by the mosquito, *Culex fatigans* (*Culex quinquefasciatus*), which has fed on infected persons. This was confirmed by Ashburn and Craig. The incubation time in the experimental disease was between three and four days. Cleland and Bradley[3] have reported experimental dengue in four non-immunes from bites of the infected females of *Aedes egyptica* (*Stegomyia fasciata*). Recently

Siler, Hall, and Hitchens[4] have reported elaborate experiments on the transmission of break-bone fever. Working on forty-two non-immunes these authors transmitted dengue fever successfully in twenty-five instances in eighty-three biting experiments. They found that the patient can transmit the virus on the first three days of his illness and that the virus is present in the mosquito *Aedes egyptica* eleven days after feeding on a case. The insects apparently remain infectious for the rest of their lives. *Culex quinquefasciatus* is not a transmitting agent. The virus is in the peripheral blood of the patient and can be filtered. The incubation period is four and a half to seven days. A definite immunity develops after an attack, not so long as that of yellow fever which dengue resembles somewhat.

We know little of the infectious agent in dengue fever. Spirochetes have been described in the blood by Couvy, but this has not been confirmed. Harris and Duval[5] have transmitted the virus to guinea-pigs, filtering it through a Berkefeld V and N. They found minute organisms like the globoid bodies of Flexner and Noguchi in poliomyelitis. These measured 0.1 to 0.3 $\mu$ in size, occurred singly, in pairs, and in short chains, resembling a short streptobacillus. They were best stained by Giemsa and by carbol-fuchsin.

Harris and Duval[6] brought these organisms to cultivation in ascitic fluid containing pieces of sterile rabbit kidney covered with a layer of oil. A turbidity developed about the pieces of kidney, spreading to within 2 to 3 cm. of the oil. Growth also occurred in Noguchi's semifluid leptospira medium as round grayish pin-point colonies about 0.3 mm. in diameter. The cultures were virulent to guinea-pigs, causing a reaction like that from dengue blood with a leukopenia and a pyrexia up to 104° to 106° F., lasting three to five days.

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## PAPPATICI FEVER

Pappatici fever or pappatasi fever is a disease occurring in the summer months, from July to the end of September, in Herzegovina, Dalmatia, and Istria, on the coast of the Mediterranean Sea. It is also called phlebotomus fever, three-day fever, and dog-fever possibly because it occurs during dog days. After an incubation period of about five days the development of the infection is rapid, with a high fever, nosebleed, severe pains in groups of muscles and along the nerve trunks, and gastro-intestinal disturbances like diarrhea. The fever lasts three days, terminates abruptly, and is followed by a long convalescence.

Pappatici fever is due to a filtrable virus as first shown by Doerr,[1] who transmitted it to other persons by the blood of patients. The virus or infectious agent is in the blood and blood-serum only on the first day, not on the second. It passes Berkefeld filters (Doerr, Tedeschi, and Napolitani[2]), Reichel filters (Doerr), and Chamberland filters (Birt[3]), but not "Pukal filters" (Doerr and Russ[4]). The quantity of blood which has been used to produce the disease varies from 0.001 to 3 c.c. The blood remains infectious "in vitro" for three and a half days (Doerr) to seven days (Birt). The artificial disease has an incubation period of three and a half to eight

days. No micro-organisms have been found in the blood either by staining with Giemsa and other dyes or by dark field illumination. The blood is not infectious to animals, dogs, guinea-pigs, rabbits, or apes. The virus is not destroyed by trypan-red or saponin.

Phlebotomus fever is transmitted from person to person by the sandfly, *Phlebotomus pappatissii*. Taussig first noted the association of this fly with cases of the disease. Doerr proved the transmission experimentally by allowing the female Phlebotomus to feed on infected individuals and later to bite non-immunes, both in regions where the disease is prevalent and in eight persons in Vienna where the infection does not occur. The insects must feed on cases in the first twenty-four hours and bite after six to seven days and up to ten days in order to carry the infection. The insects do not produce the disease by biting for thirty to forty hours after feeding, so the virus must multiply in the body of the insect. There are about fifteen species of Phlebotomus, only the female of which suck blood. The virus does not survive in flies over the winter months. It may possibly be transmitted by the female to the eggs, but this has not been proved experimentally. The disease is not conveyed by contact, and persons in contact with cases but not bitten by the flies do not develop it.

After an attack of pappatici fever a profound immunity develops, due to viricidal substances in the blood. Doerr and Russ found that 1 c.c. of serum from a man who had had an attack two years previously neutralized 0.5 c.c. of highly virulent blood.

Observations similar to those of Doerr on pappatici fever have been carried out by Birt for the simple continued fever of Malta and Creta. Birt produced this disease experimentally in London by the use of infected flies from Malta. Birt's virus passed through Chamberland Pasteur filters. Tedeschi and Napolitani have reported analogous facts in Parma for the Italian "summer fever." It seems probable that there are a number of diseases called three-day fever, six-day fever, and seven-day fever which have the same etiology and transmission.

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## JAPANESE FLOOD-FEVER

Japanese flood-fever, flood-river fever, tsutsugamushi disease, or Kedani disease is an infection which is widely prevalent in certain regions in Japan, especially in flooded districts on the banks of the rivers and on the islands. It is common on the west coast of Honshu, the principal island of Japan. In man it is characterized by continuous fever, swelling of the glands, and an exanthem. The incubation period is five to twelve days. After an attack an immunity develops which lasts several years and then disappears. The mortality is high, from 25 to 30 per cent. The disease prevails from the end of June to the beginning of October. In infected districts apes, guinea-pigs, rats, and mice are also infected.

Kitasato[1] in 1892 found that the disease is transmissible to apes by the injection of blood and that the virus can be carried from ape to ape, and from

man to man. In the experimental disease in apes the incubation period is seven to ten days, but the animals do not develop an exanthem like that in man. The virus can also be introduced into field-mice (*Micronius montebelli*) and guinea-pigs, which show no symptoms, but harbor it in the body. Injections of the spleen of these animals produce the disease in apes. The virus resists a temperature of 45° C. fifteen minutes, is destroyed by water or glycerin, and does not pass Berkefeld filters.

It has long been known that tsutsugamushi is related to the bites of insects. In 1904 Miyajima and Asakawa showed that tsutsugamus mites, known locally as akamushi or red bug, are insect carriers of the disease. These mites are the larvæ of a minute red trombidium, *Leptus akamushi Brumpt*, and are like *Leptus autumnalis*. The infection follows bites of these mites, only some of which are infected in nature. The field-mouse is apparently the natural host of the disease.

A number of observations have been made regarding the infectious agent in flood-fever. Kitashima and Miyajima[2] found small elliptical bodies in the larvæ and in the adult trombidium, cultivated them in ascites agar, and produced the disease in monkeys. Nagayo[3] has observed small oval or elliptical structures, 0.35 to 1 $\mu$ in size, with a definite limiting membrane, in the lesions in man and monkeys. Hayashi has seen minute bodies, rod-shaped, spheroid, and ring-shaped, in the lymphocytes of the lymph-nodes, in the mononuclear endothelial phagocytes of the spleen and lymph-nodes, in the region of the bites, and also free in the blood-plasma. They looked a little like *Bartonella bacilliformis* of Oroya fever and like *Theileria parva* of cattle anemia, and have been called *Theileria tsutsugamushi*. Ishiwara and Ogata[4] have observed coccoid bodies 0.2 to 2.15 $\mu$ in size in sections from the lymph-glands, spleen, and heart in cases in man and in the testicles of monkeys experimentally infected. They stained by various dyes, including Giemsa. These organisms were grown in a special ascites medium and the cultures were virulent to monkeys.

Sellards[5] has cultivated a Rickettsia-like organism from experimental tsutsugamushi disease in guinea-pigs and monkeys. These organisms were brought to cultivation four times, three times from the spleen of guinea-pigs and once from the blood of a monkey. They were grown first on chocolate agar, later on egg medium, where they produced a yellowish-brown pigment, on hydrocele agar, on whole guinea-pig blood, and on the N N N medium prepared with rabbit blood for the cultivation of Leishmania. Sellard's organisms looked like the Rickettsia of typhus fever. They stained blue with methylene-blue, pink to purple with Giemsa. Morphologically they were usually rods measuring 0.3 by 1.3 $\mu$, but very thin forms occurred, together with long, wavy, granular chains of irregular outline. They were non-motile and killed at 55° C. in ten minutes. The cultures were somewhat virulent to monkeys and highly virulent to guinea-pigs. In the latter they caused lesions just like those from the virus, including hemorrhages. Sellards regards these organisms as special types of bacteria. They have not thus far been found in patients or in the arachnids which convey the infection. Sellards' organisms have been named *Rickettsia nipponica*.

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## POLIOMYELITIS

Poliomyelitis, or infantile paralysis, is a disease of children one to four years of age and not uncommon in older children and adults. It begins suddenly with gastro-intestinal symptoms, vomiting, diarrhea or constipation, and excessive sweating. Paralysis then sets in and varies greatly in severity and distribution, affecting one or both arms and legs, the muscles of the eyes and face, the bladder, and the respiratory system. The disease regresses, leaving usually certain muscles affected. The mortality is about 10 per cent. It was first carefully studied by von Heine in 1840, and by Medin in 1887. It is often called the Heine-Medin disease. Wide-spread epidemics have occurred in Norway and Sweden, Germany, Austria, and America.

Pathologically poliomyelitis shows chiefly inflammatory foci and small-celled infiltration in the nervous system. Krause and Bonhoff have described inclusions in the glia cells.

The virus of poliomyelitis can be transferred to apes, as was demonstrated the same year by Flexner and Lewis[1] and by Landsteiner and Levaditi.[2] It is present in the nasopharyngeal mucosa and may be filtered through Berkefeld filters. To produce the disease the infectious material may be introduced into the brain, spinal cord, nerve sheaths, or peritoneal cavity. Flexner and Amoss[3] have shown that the virus can penetrate the uninjured retina without producing injury and reach the nervous system by this route. Clark and Amoss[4] have produced a regular infection of *Macacus rhesus* by intraspinous injection of the virus which passes from the subarachnoid space into the nervous tissues. Infection can usually be produced in apes by introduction of the virus into the nasopharyngeal passages. After an attack the virus persists in the nasopharynx of the monkey (Flexner and Amoss[5]).

In experimental poliomyelitis the animals show an increased irritability and a characteristic paralysis like that in man. The incubation period is five to six days, usually nine days, and may be longer, twenty days. Immunity develops after one attack of the experimental disease.

The virus has also been transmitted to rabbits by Krause and Meinicke[6] and Lentz and Huntemüller.[7] According to Marks[8] the virus can be propagated in the central nervous system of rabbits. Rosenau and Havens[9] believe that the virus can be passed from the experimental disease in monkeys into rabbits, 40 per cent. developing the disease.

In man infection apparently arises from the impingement of the virus on the injured or uninjured pharyngeal mucosa. The portal of entry is thus usually the nasopharynx, but the gastro-intestinal symptoms suggest that the intestinal tract may also admit the virus to the body. The virus is present in the nasopharyngeal passages during the disease and may persist some time (Flexner and Amoss). Taylor and Amoss[10] have found it in the nasopharynx in a child who had recovered from a non-paralytic attack previously. It has been demonstrated by Flexner and Fraser in the nasal washings of the parents of a case, who were thus passive carriers. It has also been found by Taylor and Amoss in the nasopharynx of a child who subsequently developed the disease. The nasopharynx thus serves as both portal of entry and portal of exit. The virus is not present in the blood except to a limited extent in the first seventy-two hours of the disease. The virus may apparently pass into the intestinal tract, survive the action of the gastric juice, and appear in the dejecta. Amoss and Taylor[11] have shown that the washings of the nasal and pharyngeal mucosa can neutralize the virus, this power being diminished in inflammatory conditions of the mucosa.

Outside the animal body the virus of infantile paralysis survives two to six years in glycerin, one year in 0.5 per cent. phenol (Flexner, Clark, and Amoss[12]). It survives in an ascitic fluid kidney medium twenty days, but not thirty days (Flexner and Amoss[13]). It resists drying seven days in a desiccator. It is destroyed at 25° to 50° C. in one-half hour.

Many attempts have been made to cultivate the virus of poliomyelitis, the most successful being those of Flexner and his associates. Flexner and Lewis have noted that when ascites fluid is mixed with filtered virus from the nervous system and kept at 37° C. it becomes turbid. Later these authors used sterile ascites fluid or brain extract containing a piece of rabbit's kidney mixed with 2 per cent. agar and covered with paraffin oil. Small colonies develop in the depths, but not at the surface, giving a turbidity to the tubes. These colonies are made up of spherical or almost spherical bodies 0.15 to 0.3 $\mu$ in diameter, arranged singly, in pairs, in short chains, and in small masses. They stain blue or violet by Giemsa, seldom red, and retain Gram's stain. The cultures from human material were infectious in the third generation, from ape material in the fifth, sixth, eighteenth, and twentieth generations, producing typical poliomyelitis in apes. The structures found by Flexner were termed "globoid bodies."

Globoid bodies have also been obtained in cultures by Amoss,[14] Smillie,[15] and by Heist, Solis-Cohen, and Kolmer.[16] Similar bodies have been found by Noguchi in sections of nerve tissues stained by special methods, and by Amoss[17] in the incubated brain tissue of infected monkeys.

Following an attack of poliomyelitis in man the affected individuals develop an immunity which is apparently lifelong. Wickman, in his extensive investigations, found no second attack. In the experimental disease in monkeys a profound immunity likewise develops. The blood of apes and of man contains immune substances which are capable of neutralizing the virus. These are present in man two years after recovery.

The mode of transmission has not been made out with certainty. The disease is apparently contagious and transmissible from person to person. Some evidence has been brought to show that the virus can survive for a time on the domestic fly, *Musca domestica*, and on the biting fly, *Stomoxys calcitrans*, but fly transmission of poliomyelitis has not thus far been demonstrated experimentally.

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## THE AUSTRALIAN X DISEASE

An encephalomyelitis in Australia has been described by Cleland and Campbell[1] as the X disease. The symptoms are somewhat like those of poliomyelitis, but the mortality is higher. Braine has produced a disease in monkeys by the spinal fluid from the Australian X disease. This looked a little like experimental poliomyelitis. A second case was induced in monkeys by the brain of the first case. Cleland and Campbell have conveyed the Australian X disease to monkeys, from monkeys to monkeys, monkeys to sheep, sheep to sheep, sheep to monkeys, and from monkeys to horses. Rabbits are resistant. The virus of poliomyelitis has not yet been found in the cerebrospinal fluid and the disease has been transmitted only to monkeys, not to sheep, calves, or horses. Flexner[2] regards the disparity in the action of the viruses of poliomyelitis and the Australian X disease as so great as to indicate that the two diseases are probably different unless the Australian X disease be an exalted form of poliomyelitis.

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## LETHARGIC ENCEPHALITIS

Lethargic encephalitis, often called "sleeping sickness," is a peculiar disease of man characterized by the greatest diversity of symptoms and severity. It was first described by Economo in Vienna. The disease has often been associated with epidemics of influenza, but the connection between the two diseases is by no means definite. Thus the Dublin epidemic of encephalitis in 1922–23 was not associated with influenza.

Encephalitis has been somewhat wide-spread in Europe and in America during the past few years. It is more prevalent in persons over twenty years of age. The onset is usually gradual, with lassitude, fever, muscular pains, vertigo, and sometimes vomiting as the principal symptoms. Various muscles then become paralyzed, especially those of the face, eyelids, or palate. The mortality is high, about 29 per cent. (Smith[1]).

A virus was first obtained from encephalitis by Loewe, Hirschfeld, and Strauss,[2] who employed material from the nasopharyngeal mucosa and human brain tissue, filtered it through Berkefeld filters, and inoculated monkeys subdurally and intracranially. The animals developed an encephalitis which was regarded as identical with that in man. Subsequently the virus was transferred from monkey to monkey and from monkey to rabbits. About 50 per cent. of the rabbits used were resistant to the virus. In the others a disease appeared which could be carried on in rabbits by washings of the nasopharynx, by cerebrospinal fluid, and by filtrates of the brain. Later Levaditi and Harvier[3] injected monkeys and rabbits intracerebrally with emulsions of the brain tissue from a human case. They obtained a virus in rabbits which could be passed from rabbit to rabbit. After seven passages the virus was virulent also to guinea-pigs and monkeys. It was filtrable, resistant to glycerol, and could not be cultivated by ordinary methods. In one instance rabbits were infected from the nasal secretions of a human case. Doerr has also gotten a rabbit virus from the cerebrospinal fluid and from the central nervous system tissues in man. Schnabel[4] has reported a successful transmission of a virus from the cerebrospinal fluid to rabbits by the subdural route. The exact relationship of the viruses which have been obtained from encephalitis material by the inoculation of rabbits is not clear, since viruses may also be obtained from herpes lesions which cause a similar

symptom-complex in rabbits. The encephalitis virus and the herpes virus, moreover, sometimes give a complete reciprocal immunity (Doerr and Schnabel,[5] Flexner[6] and Amoss).

Loewe and Strauss[7] have reported the cultivation of minute coccoid bodies by the Noguchi method from human cerebrospinal fluid, and filtrates of the nasopharyngeal mucosa from the brains of rabbits and monkeys artificially infected. These bodies were filtrable and resembled Flexner's globoid bodies in poliomyelitis. Cultures were virulent to rabbits and monkeys.

Levaditi, Harvier, and Nicolau[8] have described intranuclear bodies in the nerve-cells of rabbits dead of their lethargic encephalitis virus, called neuro-corpuscles. They resemble the bodies seen by Lipschütz[9] and later by Goodpasture and Teague[10] in natural and experimental herpes. Some doubt, therefore, has been thrown on the reported instances of the transmission of encephalitis to animals. Moreover, Blanc and Caminopetros[11] and Levaditi, Harvier, and Nicolau[12] found that the brains of rabbits inoculated with encephalitis material produce corneal herpes and keratitis.

Parker[13] believes that the viruses of herpes and lethargic encephalitis are different since intranuclear inclusions which are characteristic in herpes and always present in natural and artificial lesions cannot be found in encephalitis material. This is not accepted as a differential criterion by Goodpasture.[14]

It is possible that there are two viruses, one from all varieties of herpes, and the other a true encephalitis virus. The herpes virus may gain access to the brain in encephalitis and account for some transmissions, especially those to rabbits. Levaditi, Harvier, and Nicolau believe that the viruses of lethargic encephalitis and herpes are identical, varying only in degree of virulence. Kling, Davide, and Liljenquist[15] claim that they have succeeded in transmitting a true encephalitis virus to rabbits by the use of cerebrospinal fluid, emulsions of brain tissue, filtrates of nasopharyngeal and intestinal washings. The disease in rabbits was chronic, requiring weeks and months to develop. Material from the rabbit's brain does not induce keratitis in rabbits, so the virus cannot be an herpetic virus. It has not yet been shown definitely that Kling's virus is the virus of encephalitis in man.

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## HERPES

Several different lesions occur on the skin of man in which characteristic vesicles develop, and they are named usually according to their location. The simplest type is called *herpes febrilis* or *simplex;* when on the face, *herpes facialis;* on the lips, *herpes labialis;* on the genitalia, *herpes genitalis.* The more extensive type which is apt to occur on the body, along the ribs, is called *herpes zoster.* All varieties of herpes have about the same anatomical changes and usually run the same clinical course, terminating spontaneously.

*Herpes facialis* was first transmitted to the rabbit's cornea by Grüter,[1] his observations being confirmed by Kraupa[2] and Löwenstein.[3] Material from the vesicles nearly always produces a purulent conjunctivitis and keratitis in the rabbit, and can be transferred from animal to animal in series. Löwenstein found that the virus did not pass porcelain filters. Later Luger and Lauda[4] got some positive filtrations with Berkefeld filters, and Blanc and Caminopetros[5] filtered the virus through Chamberland L1. An herpetic virus was transferred to guinea-pigs also by Doerr and Vöchting[6] and by Luger and Lauda.[4] Luger and Lauda[4] and Lipschütz[7] have described intranuclear acidophilic bodies, especially in the epithelial cells, in herpes in human beings, and in experimental herpes in the rabbit's cornea. Similar bodies have been found by Goodpasture and Teague[8] in this country. They occur in the local lesions following inoculation of herpes febrilis on the conjunctiva, retina, buccal mucosa, skin, trachea, liver, adrenal, ovary, testicle, brain, and spinal cord. These inclusions are large, red, homogeneous, round or oval, finely granular; they almost fill the nucleus, but are separated from it by a clear zone. They have an affinity for acid dyes, staining with eosin in eosin-hematoxylin, after fixation in sublimate alcohol or Zenker. These authors regard the presence of intranuclear bodies as indicating the presence of the virus, and the bodies represent the growth of the virus within the affected nuclei.

*Herpes zoster* also has a virus transmissible to rabbits which is generally believed to be the same as the virus from herpes simplex or herpes febrilis. Lipschütz[7] claims that the herpes zoster virus can be successfully inoculated on the rabbit's cornea, producing purulent conjunctivitis and keratitis, but this could not be confirmed by Cole and Kuttner.[9] Intranuclear inclusion bodies have likewise been described in the lesions of human herpes zoster and in the rabbit lesions.

Teague and Goospasture[10] have produced in guinea-pigs and rabbits an experimental disease like herpes zoster in man by inoculating the tarred skin with the virus of herpes simplex. They have further shown that in intermediate forms of herpes in man, where the eruption is definitely neural in distribution, the virus is highly pathogenic for rabbits. Such a virus produces typical herpes zoster in these animals.

Blanc and Caminopetros[11] have pointed out that herpes genitalis also has a virus which produces disease in rabbits.

The virulence of the herpes virus varies greatly. While it frequently produces only local lesions on the rabbit's cornea, Doerr and Vöchting[6] have shown that some rabbits inoculated with herpetic material on the cornea developed cerebral symptoms and subsequently died. Blanc and Caminopetros[5] noted the same phenomenon independently, and showed that rabbits died with cerebral symptoms after intracerebral inoculation with herpetic material from the rabbit's cornea. According to Da Fano[12] the herpes virus can invade and localize in the central nervous system, causing a wide-spread small-celled infiltration, intense nerve-cell degeneration, and a diffuse proliferation of fixed elements. This may be regarded as an her-

petic meningo-encephalitis which is closely similar to the lesions in lethargic encephalitis and practically identical with the lesions in rabbits induced by the inoculation of encephalitis material.

Goodpasture and Teague[13] and Goodpasture[14] have found that their herpes virus, which is highly virulent, enters the nervous system along the pathway of the nerves from the primary focus. Within the brain and cord the virus produces an acute herpetic lesion with a definite relation to the nerve through which it has entered. The virus traverses sensory, motor, or sympathetic nerves. Goodpasture and Teague believe that the virus passes along the axis-cylinders rather than the perineural sheath, not passively, but by invasive proliferation.

Cross immunity experiments indicate that herpes virus and some of the so-called encephalitis viruses are either identical or closely related.

It has already been noted that Parker[15] differentiates herpes virus from encephalitis virus by the absence of intranuclear inclusions in encephalitis lesions. Goodpasture[14] has recently pointed out that the presence of herpetic inclusions cannot be regarded as a differential criterion between encephalitis and herpes, since only early cases of encephalitis would show them. Goodpasture has further shown that normal rabbits may contract herpetic encephalitis from contact with rabbits inoculated on the cornea with herpes simplex. Furthermore, rabbits can be successfully immunized against herpetic viruses.

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## TYPHUS EXANTHEMATICUS

Typhus exanthematicus, spotted fever or jail fever, has been known for years and was first clearly differentiated from typhoid fever (*Typhus abdominalis*) by Gerhardt, his observations being confirmed by Murchison. This disease was wide-spread in early times in Europe and America, but died down markedly during the last century with the inauguration of modern sanitation, remaining, however, as a prevalent affection in Eastern Europe and in Russia. During the last war typhus appeared in many areas in Europe, especially in Poland.

Typhus fever is characterized by fever, an exanthem, and symptoms referable to the nervous system. In severe cases petechiæ are abundant on the skin, with hemorrhages and gangrene. It is therefore sometimes called petechial fever. The incubation period varies from ten to fourteen days to three weeks. After a sudden onset the affection develops with high fever and general symptoms. In three to six days the exanthem appears. The disease terminates by crisis and leaves behind a profound immunity.

The specific lesions of the disease are found in the smallest arteries of the body, especially in the skin and in the nervous system. The endothelium is swollen and separated from the intima. Hyaline thrombi occur with a cellular infiltration outside the wall of the vessels. These lesions to the blood-vessels result in hemorrhages, heart weakness, and gangrene of the extremities (Wolbach, Todd, and Palfrey[1]). The perivascular accumulation of cells gives rise to the characteristic typhus nodules in the skin and brain.

Typhus fever was first transmitted from man to man by the blood by Matsukowsky, Yersin, and Vassal, and to animals (chimpanzees and "Halfaffen"). It was transmitted to guinea-pigs by Ricketts and Wilder[2] by intraperitoneal and intracardial injections of several cubic centimeters. The infection in chimpanzees corresponds to the disease in man. In apes and guinea-pigs fever only appears, although Löwy[3] has described an exanthem in guinea-pigs, and v. Prowazek[4] and Bauer[5] have found arterial lesions like those in man. The infection has been transmitted from animal to animal and carried twenty generations by da Rocha-Lima.[6] The virus in guinea-pigs has also been carried back to apes. The virus is present in the blood of man during the entire period of pyrexia, even in the first few days, but not during convalescence. According to Nicolle the cell-free plasma does not contain the virus, while small quantities of leukocytes are definitely infectious. Von Prowazek has described cell inclusions in the white corpuscles and believes that they are related to the development of the virus.

Typhus fever was first transmitted from man to apes by the body louse, *Pediculus vestimenti*, by Nicolle.[7] This was confirmed by Ricketts and Wilder and by Anderson and Goldberger[8] in Mexican typhus, known locally as "tabardillo." It was also confirmed later by da Rocha-Lima, who transmitted the disease to guinea-pigs by the same method. At the present time *Pediculus vestimenti* is regarded as the chief insect vector, although *Pediculus capitis*, the head louse, may also transmit the infection (Anderson and Goldberger).

The louse becomes infected by one feeding on a case of the disease, and one bite can transmit the infection to a susceptible animal. The louse apparently serves as an intermediate host in which the parasite develops or ripens. Very small quantities of blood can infect a louse, while several thousand times this quantity, *i. e.*, several cubic centimeters, are needed to produce infection by inoculation. Furthermore, the louse becomes infectious only after four to five days, so an increase of the virus must take place in its body. Rarely the infection passes from the infected female to the young, as first shown by Ricketts and Wilder and confirmed by da Rocha-Lima. Breinl[9] has found that the virus of typhus can be carried from louse to louse, then back to guinea-pigs and rabbits. Breinl believes that it multiples in the louse, but shows no increase of virulence.

Many attempts have been made to find micro-organisms in typhus fever, both in the lesions in man and animals and in infected lice. Ricketts first described structures which might be micro-organisms in the lesions of the disease and in infected lice. Ricketts and Wilder in 1910 found oval bodies in smears from infected lice. These exhibited polar staining with central light areas like the organisms of the hemorrhagic septicemia group. Similar bodies were found by v. Prowazek in 1913, and by Sergent, Foley, and Vialatte[10] in Tunis in 1914. During the war Ricketts' bodies were especially investigated by da Rocha-Lima and by Töpfer and Schüssler,[11] who concluded that they represent the form of the virus in the louse. There is a distinct parallelism between their presence and the infectivity of the louse.

In the first four to five days after feeding, the lice are not infectious and do not show the bodies. The bodies appear as the lice become infectious. Furthermore, both these bodies and infectivity are lacking when the lice are kept at temperatures below 24° C. and when lice bite convalescents. According to da Rocha-Lima development takes place in the epithelial cells of the stomach and intestine, the bodies increasing so much that the epithelial cells are filled, balloon out into the lumen of the alimentary canal, and finally break and empty into the canal. The affected cells stain pale red by Giemsa, while normal cells have a blue plasma. Sikora[12] has also found Ricketts' bodies in the salivary glands of the louse.

The bodies originally found by Ricketts and Wilder and investigated then by v. Prowazek have been termed *Rickettsia Prowazeki* by da Rocha-Lima, and considerable discussion has developed as to their nature and function. They have been investigated, especially by Wolbach[13] and his co-workers, who have found them in infected lice and in the lesions of typhus in man and guinea-pigs. In the louse they first appear as bacillary or filamentous structures in the intestinal epithelium, especially in the lightly infected cells. As the numbers increase, coccoid, filamentous, and deeply stained paired forms are to be found. Minute paired structures can be observed in the epithelial cells of the digestive tract of the louse, and these Wolbach regards as *Rickettsia Prowazeki*. In animal tissues they appear as short bacillary and diplococcoid forms in the endothelial cells of the blood-vessels.

*Rickettsia Prowazeki* measures 0.25 to 0.35 $\mu$ in width and from 1 to 2.5 $\mu$ in length. The filamentous or thread forms are usually curved or flexed, 0.3 to 0.4 $\mu$ in width, and 10 to 40 or 50 $\mu$ in length. In the short forms the polar granules stain deep red, the intervening portions light clear blue. The filamentous forms stain blue.

Arkwright, Bacot, and Duncan[14] have shown that the louse which commonly infects *Macacus rhesus*, *Pedicinus longiceps*, becomes infected with *Rickettsia Prowazeki* when fed on typhus monkeys. Intranuclear Rickettsias do not occur normally in this species. Atkin and Bacot[15] have further shown that *Pedicinus longiceps*, which harbor *Rickettsia Prowazeki* after feeding on typhus-infected monkeys and after rectal injections of typhus blood, are virulent to monkeys.

Attempts to cultivate *Rickettsia Prowazeki* have not been successful, but are not without interest. Weigl[16] has devised a method of feeding lice by the anus and has infected normal lice with *Rickettsia Prowazeki* by injecting leukocytes from typhus patients and from typhus-infected guinea-pigs. This was confirmed by Bacot and Ségal[17] and by Arkwright and Bacot.[18] The typhus virus which had been carried in guinea-pigs more than a year would always produce Rickettsias in lice, and these Rickettsia-laden lice would always infect guinea-pigs if emulsions were made and injected.

Wolbach and Schlesinger[19] have made tissue plasma cultures from typhus material in infected guinea-pigs in which the virus has been kept over three years without intervention of lice. Within the cells of endothelial origin they found bodies with all the characteristics of *Rickettsia Prowazeki*, filamentous, short, bacillary, and diplococcoid forms. These cultures produced typhus in guinea-pigs and remained infective as long as the tissue cells of the culture survived. This period was prolonged by transferring the material to fresh plasma.

According to Wolbach the principal points in the evidence that *Rickettsia Prowazeki* is the causative micro-organism of typhus fever are:

1. The occurrence in lesions.

2. The occurrence in lice as the sole distinctive acquisition when the lice are fed on typhus patients.
3. The failure to separate them from the virus by any method employed.
4. The cultivation in lice by the method of intestinal tract injection.
5. The cultivation in tissue plasma cultures.

A cultivation of Rickettsias has also been claimed by Loewe, Ritter, and Baehr from blood of typhus fever patients and from the brains and kidneys of guinea-pigs reacting to typhus blood. These workers used rich ascitic fluid containing a fragment of rabbit or guinea-pig kidney and 2 per cent. dextrose broth, covered with petrolatum.

The serum from typhus fever patients agglutinates various bacteria, particularly strains of *Proteus vulgaris*. This reaction has been investigated especially by Weil and Felix[20] and is known as the Weil-Felix reaction. Two strains of Proteus, Proteus $X_2$ and Proteus $X_{19}$, have been found serviceable. They were first obtained from the urine of typhus patients.

After an attack of typhus fever man becomes immune and a similar immunity follows in animals after the experimental disease. Otto[21] claims that Rickettsia bodies are agglutinated specifically by the serum of patients with typhus.

The filtrability of the typhus fever virus has been much discussed. Ricketts was unable to produce infection in animals with material passed through Berkefeld filters. Nicolle, Comte, and Conseil[22] believe the virus to be filtrable. After inoculation with filtrates of typhus blood or crushed infected lice monkeys showed no decided rise in temperature, but became resistant to injection of the unfiltered virus. Olitsky[23] has been unable to filter the virus in blood or in disintegrated tissues of infected guinea-pigs through Berkefeld V and N. He found, however, a substance in filtrates from infected tissues which produce a thermic reaction and an immunity in animals. At the present time filtration of the virus through bacteria-tight filters is not usually accepted.

From time to time cases of typhus fever are reported in America. The cases in New York City were studied especially by Brill,[24] who believed that they represented a distinct type of disease. Hence they are often called Brill's disease. It is now believed that some of the cases of Brill's disease in New York were examples of European typhus fever, since they occurred largely in the families of immigrants recently arrived in America from eastern Europe. Anderson has succeeded in transmitting a virus from Brill's disease in New York to apes, the animals developing the characteristic symptom-complex of experimental typhus fever. In other instances there is no evidence that the persons with typhus fever or Brill's disease have ever come in contact with European immigrants or are likely to have been bitten by lice from known cases. The etiology of these cases is doubtful. Possibly we still have unreported or unrecognized typhus fever in this country, possibly an animal reservoir of the infection. Some of the cases reported as typhus fever or Brill's disease may also be examples of some other disease with a symptom-complex like typhus fever, but of milder aspect.

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## TRENCH FEVER

Trench fever, Wolhynian fever, or five-day fever is an acute febrile affection which was widely prevalent in the armies during the late war. It was especially investigated by Graham[1] and Herringham[2] in the British Army in France, and by His[3] and Werner[4] in the German Army in Poland and Wolhynia. The disease is characterized clinically by sudden onset, fever, headache, and pains in the muscles. Later the cases have pain and tenderness in the joints and a peculiar remittent fever (described by Swift as spiky). The patients are subject to frequent relapses. The mortality is negligible, the disease owing its importance to the disability it may cause in large numbers of soldiers.

Trench fever was transferred from man to man by McNee and Renshaw[5] by the intravenous and intramuscular injection of whole blood. The virus is in or attached to the red cells and does not pass Berkefeld filters. It was later shown by the American Commission,[6] with Strong as chairman, that the virus is also in the plasma. The urine of patients likewise contains the virus and in this medium it can be filtered through Berkefeld candles. The American Commission further showed that trench fever can be conveyed by the bites of lice fed on infected persons. In their experiments 18 out of 23 persons contracted the disease. The incubation period varied from fourteen to thirty-eight days and the experimental infection was practically the same as the natural.

These observations were confirmed by the British Commission,[7] which also showed that the excreta of infected lice can produce the disease when applied to the scarified skin, *i. e.*, the virus is in the dejecta of the lice.

Byam and Lloyd[8] state that trench fever patients can infect lice three hundred to four hundred days after the onset of the disease. The virus is probably not transmitted through the eggs of the lice.

Rickettsia bodies have been found in lice infected from trench fever by Töpfer,[9] by da Rocha-Lima,[10] and by Jungmann and Kuczynski.[11] They are extracellular, slightly plumper and more oval than *Rickettsia Prowazeki*. These extracellular Rickettsia are known as *Rickettsia pediculi*, *Rickettsia*

*Wolhynica*, and *Rickettsia quintana*. There has been a good deal of discussion as to whether they represent the virus or not. Munk and da Rocha-Lima[12] have reported similar organisms in presumably normal lice. Strong[13] regards trench fever as due to a filtrable virus and not to Rickettsias. Arkwright, Bacot, and Duncan[14] believe that the trench fever virus in the louse is always accompanied by these extracellular Rickettsias. Bacot[15] contracted trench fever after a visit to a public bath in Warsaw during which he picked up lice. Lice free from Rickettsias were allowed to feed on him; after eight days they showed extracellular Rickettsias in great numbers, none intracellular. Bacot continued to infect fresh lice for three months after the disappearance of all symptoms.

The relationship of *Rickettsia pediculi* to trench fever cannot be regarded as settled at present. Lice in which Rickettsias have not been demonstrated sometimes produce trench fever when fed on man, as well as the lice with Rickettsias. Thus far Rickettsias have not been found in the blood of patients. The pathology of the disease has not been worked out.

Sikora[16] has cultivated *Rickettsia pediculi* in the celomic cavity of the louse and in human blood agar at 37° C., not on horse blood agar or rabbit blood agar. It grew best on Nöller's medium, blood agar inactivated at 57° C.

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*See also* Wolbach and Todd in the Report of the Typhus Research Commission and Wolbach's Typhus and Rickettsia in Harvey Society Lectures, 1920–21, p. 188.

## ROCKY MOUNTAIN FEVER

Rocky Mountain fever, or Rocky Mountain spotted fever, is a peculiar disease which has prevailed in certain areas in the United States since early times and has shown little or no marked tendency to spread to other regions. It occurs chiefly in the Bitter Root Valley and in the Lo Lo Valley in Montana. It is characterized by abrupt onset, high fever, up to 102° to 104° F., a generalized rash which is later hemorrhagic, and violent nervous symptoms. The mortality varies from 7 to 13 per cent. Cases of Rocky Mountain fever have also been reported from Nevada, Wyoming, California, Colorado, Washington, and Idaho (Snake River Valley). Wolbach[1] has studied the lesions in man, monkeys, guinea-pigs, and rabbits, and finds that it is essentially an acute, specific, infectious endangeitis with an accumulation of vascular endothelial cells, necrosis, and thrombosis. These lesions occur chiefly in the skin and in the genitalia.

In Rocky Mountain fever a virus is present in the blood-serum and corpuscles which does not pass Berkefeld filters. This virus produces the disease in guinea-pigs, rabbits, and apes.

The association of attacks of Rocky Mountain fever with the bites of the wood tick, *Dermacentor venustus*, was first noted by Wilson and Chowning.[2] Subsequently Ricketts[3] produced the infection in guinea-pigs by allowing wild ticks to feed on them. The eggs of infected female ticks are infectious and both male and female transmit the disease. The lesions in guinea-pigs are marked swelling and reddening of the skin of the scrotum, of the eyelids, ulcers of the paws, and a general febrile reaction. At autopsy there is edema and hemorrhage of the skin and subcutaneous tissues of the scrotum. Rabbits are occasionally susceptible (Foot[4]). The disease can be transmitted to monkeys which die after seven days.

Structures believed to be micro-organisms were first observed in infected blood by Ricketts and described as lanceolate chromatin-staining bodies. These have been investigated especially by Wolbach, who believes that they belong in the general group of *Rickettsia*. In infected ticks they occur as: (1) Intracellular bacillary forms without chromatoid granules, present only in the cytoplasm of the cells of the alimentary tract; (2) small rod-shaped structures with chromatoid granules inside cells and inside nuclei; (3) large lanceolate paired bodies persisting in the tissues and especially in the salivary glands long after the others have disappeared and characterized by their chromatoid staining. This last form is the only one found in the eggs of infected ticks.

The bodies occur also in the nymphæ raised from the eggs. Infected ticks always harbor these pleomorphic structures which are also present in the lesions of the disease in man, monkeys, guinea-pigs, and rabbits. Here they occur chiefly as minute diplococcus-like bodies in the endothelial cells of the vascular lesions, in smooth muscle cells, and occasionally in detached endothelial cells. They stain with eosin-methylene-blue, and with Giemsa. They are not mitochondria, cytoplasmic or nuclear granulations (Cowdry[5]). Wolbach's observations have been confirmed by Nicholson.[6] The Ricketts bodies have been named *Dermacentroxenus Rickettsii* by Wolbach.

Bodies similar to those observed by Ricketts, Wolbach, and Nicholson have been found by Connor[7] in the blood and in the cell-free plasma after prolonged centrifugation. They look like pleomorphic bacteria. They stain blue by Giemsa and have a shadowy outline without the clear-cut borders of most bacteria. They may be bacillary, lanceolate, diplobacillary with flat ends, joined by a non-staining material. The free ends are more pointed than those of the pneumococcus. The structures may show solid staining or one or two chromatoid bodies. They are best obtained by recovering infected blood from the peritoneum of guinea-pigs two days after intraperitoneal inoculation.

Several attempts have been made to cultivate the Rickettsias in Rocky Mountain fever; the most important are those of Wolbach and Schlesinger.[8] These observers made tissue plasma cultures from infected guinea-pig tissues and normal plasma. They found that the Rickettsias multiplied in the endothelial cells of the blood-vessels of the tissue and later in endothelial cells that had wandered out into the plasma. The cultures were maintained four weeks and were infectious as long as the cells were kept alive by transferring to fresh plasma.

Wolbach regards the principal arguments for regarding *Dermacentroxenus Rickettssii* as the causal organism in Rocky Mountain spotted fever as:

1. Constant occurrence in the lesions in man, monkey, rabbit, and guinea-pig.
2. Morphological sequence in ticks.
3. Cultivation in virulent form in tissue plasma cultures.

A potent immune serum against the virus of Rocky Mountain fever has been produced by Noguchi.[9] Its early administration, within the incubation period, prevents the development of the disease in guinea-pigs.

Parker, Spencer, and Francis[10] have recently shown that ticks may harbor the virus of Rocky Mountain fever as well as the organisms responsible for tularemia, *Bacterium tularense*. This suggests that there may be a reservoir of the disease in nature from which the ticks get the infection, possibly rabbits or gophers.

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## VERUGA PERUVIANA AND OROYA FEVER

Veruga Peruviana or Veruca Peruviana is prevalent in the elevated valleys of the Peruvian Andes and is characterized by fever, anemia, and wart-like tumors of the skin and mucous membranes. It is closely associated with Oroya fever, with which it is identified by some authors. In Oroya fever the patients have fever, anemia, hemorrhages, pains in the joints, and gastro-intestinal symptoms. Small and large tumors develop on the surface of the body, especially on the face and extremities, and a vesicular and pustular eruption.

Carrion injected himself with the blood of a patient presumably having Veruga Peruviana, developed a disease, and died. The infection is therefore often called Carrion's disease, but it is not certain that Carrion did not have Oroya fever.

In Oroya fever cell inclusions have been described by Barton,[1] Biffi,[2] and Gastiaburú.[3] They are present in the erythrocytes partly as small roundish bodies and partly as short plump rods. These structures have been named *Bartonella bacilliformis* by Strong.[4] They are regarded by Mayer[5] and by Schilling[6] as degeneration products of the red cells.

Mayer, da Rocha-Lima, and Werner[7] have described masses of large spindle-shaped cells with light blue homogeneous protoplasm in which they find various cell inclusions. These vary in size from small bodies 1 to 1.5 $\mu$ up to the diameter of erythrocytes, are homogeneous or finely granular by Giemsa, are paranuclear or free. Or they may be larger, light or brick-red when stained, filling the expanded portions of the cell protoplasm. These structures are regarded as Chlamydozoa by these authors, who believe the etiological agent to be a filtrable virus.

Strong[8] and his co-workers believe that Oroya fever is usually fatal and is caused by *Bartonella bacilliformis*, which resembles the parasite of East African Coast fever and has not yet been cultivated. Inclusions occur in the endothelial cells of the blood-vessels. This disease is not transmissible to animals. The infectious material from Veruga Peruviana can be transmitted to dogs, rabbits and apes. No blood parasites or cell inclusions can be found in Veruga.

Jadassohn and Seiffert[9] found a case of Veruga Peruviana in Berne, infected sixty days previously in Peru. They transmitted the disease to apes, Cercopithecus and Rhesus, characteristic warts appearing on the eyebrows.

Townsend[10] believes that *Phlebotomus verrucarum* serves as an insect vector in Veruga Peruviana. Apes were infected by biting and dogs by injecting an emulsion of 20 female phlebotomus under the skin.

Noguchi and Battistini[11] have recently isolated an organism identified as *Bartonella bacilliformis* from a case of Oroya fever, a minute motile pleomorphic rod. It was Gram-negative and stained reddish-violet with Giemsa. It varied in width from less than 0.2 to 0.5 $\mu$ and in length from 0.3 to 2.5 $\mu$. Cultures were virulent to young rhesus monkeys, inducing an intermittent fever which lasted many weeks. Typical endoglobular forms of *Bartonella bacilliformis* were present in the red blood-corpuscles. Intradermic inoculation produced highly vascular nodules about the eyelids resembling the nodules of experimental Veruga found by earlier investigators.

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2. Biffi: Crón. méd., Lima, 1903, xx, 149.
3. Gastiaburú: Crón. méd. Lima, 1903, xx, 314.
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5. Mayer, M.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1910, lvi, Orig., 309–311.
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10. Townsend, C. H. T.: J. Am. M. Ass., 1913, lxi, 1717–1718.
11. Noguchi, H., and Battistini, T. S.: Science, 1926, lxiii, 212–213.

## VERRUCA VULGARIS

Common warts, or Verruca vulgaris, were shown to be transmissible by Variot, de fine Licht, and Jadassohn,[1] who obtained 33 positive takes in 74 inoculations. The incubation period varied from six weeks to eight months. No cell inclusions were found. Ciuffo[2] filtered the virus of warts through a Berkefeld N and W. It has not been cultivated.

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## MOLLUSCUM CONTAGIOSUM

Occasionally infectious epithelial tumors occur in man about the face and eyelids which undergo colloid degeneration, giving rise to peculiar "molluscum bodies." These tumors are round or oval, large, sharply circumscribed, turn yellowish-green on the application of nitric acid, and give a reaction for fat with osmic acid. It has been shown by Juliusberg[1] that the tumors can be transmitted from person to person by a virus which passes Chamberland filters. He got three positive takes after an incubation period of fifty days. In man the incubation period is about eight weeks and the infection apparently gains entrance through injured epithelium.

Lipschütz[2] states that the etiological agent consists of round or dumbbell-shaped bodies about $\frac{1}{4}$ $\mu$ in size. They are non-motile, have no membrane, and may be stained by Giemsa or Loeffler. They are called *Strongyloplasma hominis* by Lipschütz. The molluscum bodies may be reaction

products, possibly keratin, arising in response to the invasion of a specific virus.

Molluscum contagiosum of man has not been transferred definitely to animals, and is apparently different from a similar disease found in fowls, epithelioma contagiosa.

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## TRACHOMA

Trachoma is a disease limited to the conjunctiva and characterized by specific proliferation of the adenoid layer. This produces follicles which look like frog spawn and give the conjunctiva an uneven, raw appearance (*Traxus* = raw). The increase of connective tissue may lead to pannus, injury to sight, and often blindness. Trachoma is the same as the Egyptian eye disease and is wide-spread in certain areas of Eastern Europe. Several foci of the disease have been recorded in America, especially in the mountains of Kentucky and Tennessee. It is apparently transmissible from person to person by the secretions of the eye and by the handling of objects contaminated by these secretions.

Trachoma was first produced in man by Greef,[1] who employed secretions from a human case, the disease developing ten days after inoculation. It has been transmitted to apes by Hess and Roemer.[2] According to Nicolle, Cuénod, and Blaizot[3] and to Bertarelli and Cecchetto[4] the virus is filtrable, but filtration is not accepted by Pfeiffer and Kuhnt[5] or Fermi and Repetto.[6] The trachoma virus resists drying, can be preserved in glycerin up to ten days, and is destroyed at 56° C. in thirty minutes.

An immunity is left behind after recovery. Nicolle, Blaizot, and Cuénod[7] have produced an active immunity in Macacus.

In 1907 Halberstaedter and v. Prowazek[8] described cell inclusions in the epithelial cells of the conjunctiva in trachoma cases in Java. These were dark blue masses when stained by Giemsa, surrounding the nucleus and containing single or double fine, red, pointed bodies. They were subsequently found by Greef, Frosch, and Claussen[9] and by Leber.[10] They were present in the material used by Greef, Frosch, and Claussen for the production of trachoma in man. These cell inclusions have been called Chlamydozoa by v. Prowazek. They have not been cultivated.

Similar cell inclusions have been described in gonorrheal conjunctivitis (blenorrhea of the newborn), in infectious vaginal catarrh of beef, and in swine-pest (Uhlenhuth). They are not absolutely specific for trachoma and at present cannot be regarded as the cause of the disease. Other cell inclusions have been found in trachoma by Addario.[11]

Botteri[12] observed that the secretions from a fresh case of blenorrhea filtered through a Berkefeld bougie produced a severe conjunctivitis in apes (Pavian), which could not be produced by the trachoma virus. The epithelial cells contained many small-cell inclusions staining blue or red. The filtrate contained very small structures staining reddish-violet by Giemsa. It is possible, therefore, that there is a cell inclusion conjunctivitis caused by a filtrable virus which is independent of trachoma.

Typical cell inclusions have been found by Huntemüller and Paderstein in swimming-pool conjunctivitis. Noguchi and Cohen[13] also believe that the trachoma bodies are the cause of a conjunctivitis different from trachoma.

Some discussion has arisen from time to time in regard to the diagnosis of trachoma. Conjunctivitis from other causes differs greatly in severity,

and long-standing, chronic cases are difficult to differentiate from it. Williams[14] has found rods like influenza bacilli in many cases of severe conjunctivitis which have the characteristics of trachoma. In cultures these organisms show considerable similarity to trachoma bodies.

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3. Nicolle, C., Cuénod, A., and Blaizot, L.: Compt. rend. Acad. d. sc., 1912, clv, 241–243. *Ibid.*, 1913, clvi, 1177–1179.
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6. Fermi, C., and Repetto, R.: Berl. klin. Wchnschr., 1907, xliv, 1197–1199.
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*See also:* Claussen, W.: Klin. Jahrb., 1909, xxi, 505–513.
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11. Addario, C.: Arch. f. Augenh., 1900, xli, 20–37. *Ibid.*, 1909, lxiv, 265–276.
12. Botteri, A.: Wien. klin. Wchnschr., 1911, xxxiv, 1429. *Ibid.*, 1912, xxv, 231–232.
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## RABIES

Rabies (Wut, rage) or hydrophobia is an infectious disease of dogs and wolves, transmissible by biting to many other animals, including cats, horses, beef, swine, sheep, and goats. It is transmissible to man, but man is not very sensitive to the virus. The mortality varies from 10 to 15 per cent. for light wounds, to 80 per cent. for wounds of the exposed parts. Daebert states that 14.8 per cent. of those bitten contract the disease in Prussia.

The period of incubation varies greatly in different species and corresponds to the location of the bite. The nearer the bites are to the nervous system, the shorter the incubation period. In man injuries on the unprotected parts of the body, like the hands and face, are more dangerous than those on the covered parts, like the legs and body. Tearing wounds, especially those made by rabid wolves, are especially likely to produce rabies. In man the incubation period varies from fifteen to sixty days, but it may be even longer, up to one year.

The symptoms of rabies also vary considerably. In man they consist of great uneasiness, headache, cramps in muscles of deglutition and respiration, and later in nearly all the muscles of the body. Cramps in the muscles of swallowing may arise reflexly at the sight of fluids. In untreated cases death may follow in four to six days after the development of symptoms.

In animals, especially in dogs, two varieties of rabies have been noted —running rabies (rasende Wut) and dumb rabies (stille Wut).

In running rabies (rasende Wut) there is a great excitability of the motor nerves. The rabid dogs show marked irritability and an abnormal appetite, attempting to eat all sorts of objects, wood, stones, straw, nails, etc. They howl constantly, leave their ordinary places of refuge, and run about the streets and country. The flow of saliva is excessive and the animals have difficulty in swallowing from the cramps in the muscles of deglutition. They are often frightened by the sight of water. Later they show wide-spread muscular cramps in the muscles, especially in those of the jaws. Death may occur in three to four days from heart failure and is usual after one to two

weeks. In this type of rabies the animals show an excessive desire to bite, attacking man and the animals with which they come in contact, especially when running about.

In dumb rabies (stille Wut) the animals are apathetic, comparatively free from excitement, show little tendency to bite, have great difficulty in swallowing, and die of a generalized muscular paralysis.

The disease is transmitted from animal to animal by the saliva during biting.

Rabies can be produced experimentally in dogs, guinea-pigs, rats, mice, and birds. In rabid animals the virus is in the brain, spinal cord, and salivary glands, seldom in the blood. It is especially abundant in the medulla oblongata. The diagnosis in suspected cases is best established in rabbits by injecting 1 to 2 c.c. of a 5 per cent. salt solution emulsion of this either into the muscles of the back, into the brain, or into the scarified eye (Ferran). Rabbits develop typical symptoms in two to four weeks, usually dumb rabies.

In experimental rabies the virus travels from the site of the wound to the nerve centers, chiefly along the nerve sheaths.

Rabies virus resists putrefaction and the action of glycerin, and may be dried for at least nine months without destruction. It is destroyed at 52° to 58° C. in thirty minutes, at 50° C. in one hour, at 45° C. in twenty-four hours.

The virus of rabies is now regarded as filtrable as first shown by Di Vestea and Zagari,[1] later by Remlinger.[2]

Characteristic bodies have been found by Negri[3] in rabies, especially in the large pyramidal cells in the horn of Ammon, and in the cerebral cortex, and in the ganglion cells in the cerebellum. The Negri bodies are cell inclusions, round, egg-shaped, pear-shaped, varying from 1 to 27 $\mu$ in size. They are easily seen unstained or may be demonstrated in properly made films when stained by Giemsa. They can be found in sections hardened by Zenker and stained by eosin and methylene-blue, and by methylene-blue followed by tannin. They appear as minute bodies in the periphery of the cell, and round or oval bodies in the center. They vary in size and there may be twenty to thirty small ones to one large one.

Negri bodies are now regarded as diagnostic for rabies. They can be found in nearly all cases (up to 80 per cent.), and do not occur in diseases resembling rabies, like "Pseudowut." They are probably not the virus, since they are held back by filters. As pointed out above, the virus itself is apparently filtrable.

Other structures found in rabies and thought to be micro-organisms are the granulations of Babes[4] and the small black bodies of Koch and Rissling[5] and of Noguchi.[6]

Especial interest attaches to protective inoculation against rabies because of Pasteur's investigations. Pasteur[7] found that the virus, when transferred to rabbits by subdural inoculation, gradually became more virulent while the period of incubation dropped from three weeks to one week. The virus was now stationary and was called "virus fixe" by Pasteur. Pasteur dried the spinal cord over calcium chlorid at 20° to 21° C. and found that it lost virulence progressively. An eight-day cord no longer caused the disease, while a cord dried for a shorter time produced it after a long incubation period. If animals were immunized during the incubation period they were protected from the disease.

Immunization against rabies was introduced in man by Pasteur. The inoculations were carried out as soon as possible after the people were

bitten. Originally the start was made with a twelve-day virus, then a stronger virus was injected, lastly, a three-day virus. In other instances an eight-day virus was employed first, then a two-day virus. At the present time a three-day virus is often used first, then a one-day virus.

The results of immunization by Pasteur's method are remarkable. In some years the mortality is reduced to one-half of 1 per cent. It fails practically only in cases where treatment is delayed or very rarely when children are bitten on the hands or face by rabid animals which harbor a virus of excessive virulence.

A number of modifications of Pasteur's protective inoculation have been tried out, but it is not certain that they are as eminently satisfactory as the original. Thus Högyes[8] makes a dilution of the emulsions of the cord, believing the diminution of virulence in the virus is due to a destruction of micro-organisms. Harris[9] employes dried brain and cord cooled below the freezing-point; this retains its virulence eight months. Ferran[10] injects the virus into the anterior chamber of the eye in immunizing the rabbits. In America the Cumming method of preparing rabies vaccine is largely followed. This vaccine is a sterile dialyzed suspension of brain tissue from rabbits killed by fixed virus. It is standardized by weight so that 2 c.c. contain sufficient material for one injection. Fourteen doses are given in mild cases; twenty-one in severe cases, including all those with bites or lacerations about the head or face.

Dogs may be immunized against rabies by protective inoculation. The practice has not spread widely, since the protection does not seem to be lasting.

1. Di Vestea and Zagari: [Abstr.] Centralbl. f. Bakteriol. [etc.], 1889, vi, Ref., 25–27. Fortschritte der Med., 1889, vii, 241–261, 281–292.
2. Remlinger, P.: Ann. de l'Inst. Pasteur, 1903, xvii, 834–849. *Ibid.*, 1904, xviii, 150–164.
3. Negri, A.: Ztschr. f. Hyg., 1903, xliii, 507–528. *Ibid.*, 1904, xliv, 519–540. *Ibid.*, 1909, lx, 421–443.
4. Babes, V.: Presse Med., 1900, i, 202–203.
5. Koch, J., and Rissling, P.: Ztschr. f. Hyg., 1910, lxv, 85–112. *Ibid.*, 1910, lxvi, 443–454.
6. Noguchi, H.: J. Exper. M., 1913, xviii, 314–316.
7. Pasteur, L.: Compt. rend. Acad. d. sc., 1881, xcii, 159–165.
8. Högyes, A.: Centralbl. f. Bakteriol. [etc.], 1887, i, 92–94. Ann. de l'Inst. Pasteur, 1888, ii, 133–152.
9. Harris, D. L.: J. Infect. Dis., 1912, x, 369–377. *Ibid.*, 1912, xi, 397–401.
10. Ferran: [Abstr.] Ann. d. l'Inst. Pasteur, 1888, ii, 286–287.

## AUJESZKY'S DISEASE

Aujeszky's[1] disease (Pseudowut), or *paralysis bulbaris infectiosa*, occurs in dogs and somewhat resembles rabies. It has a shorter incubation period, a more rapid course, and a greater local inflammation at the site of the subcutaneous introduction of the virus. Negri bodies are not demonstrable in the lesions which are similar to those of rabies. The disease is transmissible to normal animals by a virus which is found in the central nervous system, in the blood, and in organs rich in blood. This virus can be preserved in glycerin several months, is sensitive to high temperatures, and injured by antiseptics in a short time. It has been filtered by Schmiedhöffer[2] through filters with large pores.

1. Aujeszky, A.: Centralbl. f. Bakteriol., 1. Abt., 1902, xxxii, Orig., 353–357.
2. Schmiedhöffer: Ztschr. f. Infektionskr. . . . d. Haustiere, 1910, viii, 383–405.

## DOG DISTEMPER

Dog distemper (Hundestaupe) is an acute transmissible disease of young carnivora, especially young dogs, marked by fever, acute catarrh of the mucous membranes, catarrhal inflammation of the lungs, and symptoms pointing to lesions of the nervous system. It is extremely contagious, passing rapidly to non-immunes, especially to young animals. The attacks vary greatly in severity and the mortality may be very high. At autopsy the lesions are chiefly in the respiratory system and in the nerve tissues, where there is a disseminated myelitis and poliomyelitis. One attack confers an immunity which seems to be lasting.

The etiology of dog distemper has been investigated repeatedly and yet a good deal of doubt remains as to its causation. Several different species of bacteria have been regarded as etiological agents, especially *Bacillus bronchisepticus* and *Pasteurella canis*, a member of the hemorrhagic septicemia group. Such organisms are frequently found associated with cases of the disease, but it is not certain that they are not secondary invaders.

Carré[1] attributes dog distemper to a filtrable virus in the blood and in the serous nasal secretions. His observations were confirmed by Lignières,[2] who regards the disease as different from the affection caused by *Pasteurella canis* and calls it "Hundepest." Kregenow[3] could not filter the virus.

Cell inclusions have been described in distemper by Standfuss and Lentz,[4] especially in the ganglion cells of Ammon's horn. These "Staupekörperchen" are easily differentiated from Negri bodies by their lack of internal structure and by their free position in the tissues or in the débris of ganglion-cells. They may belong to the Chlamydozoa or Strongyloplasmata of Lipschütz.

1. Carré, H.: Bull. d. Soc. centr. de méd. vét., 1905, lix, 335–339.
2. Lignières, J.: Bull. d. Soc. cent. de méd. vét., 1906, lx, 668–681.
3. Kregenow, C.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1909, l, Orig., 326–344.
4. Standfuss and Lentz: Arch. f. Tierheilk., Bd. 34.

## PERIPNEUMONIA

Peripneumonia or pleuropneumonia of cattle is an infectious disease which is wide-spread in Europe and occasionally epidemic in North America, as in 1859. It is characterized by pleurisy and inflammation of the lungs with occasional suppuration in other organs. In the lungs a serofibrinous exudate collects in the interlobular and subpleural cell tissues. The disease has a high mortality.

The contagious character of pleuropneumonia was first definitely established in 1850 by a Commission of French scientists under Dumas. Willems[1] showed that the disease is transmitted by sick animals to normal and leaves behind an immunity. He also showed that the virus is present in the exudate of lymph in the lungs and that animals can be protected against the disease by inoculation. The virus is in the secretions of the respiratory tract and in infected tissues, not in the blood.

Somewhat later Pasteur[2] found that he could preserve the virus without loss of infectivity. He could not find micro-organisms in it or multiplication in ordinary broth. Nocard, Roux, Borrel, Salimbeniet, and Dujardin-Beaumetz[3] brought the virus to cultivation in nutrient broth in collodion sacs placed in the peritoneal cavity of rabbits. After three to four weeks the sacs contained an albuminous opalescent fluid in which a number of small refractile motile bodies were present, so small that their morphology

could be made out with the greatest difficulty by microscopes giving a magnification of 1500 diameters. These bodies showed a great diversity of form, cocci, spirilla-like bodies, and star-shaped forms. The peculiar turbidity could be transferred from sac to sac in the peritoneal cavity of rabbits, but not of guinea-pigs. At the same time the virus was constantly present, a few drops producing a characteristic disease in cattle which was often fatal. The recovered animals were resistant to infection with the virus.

The micro-organisms could not be cultivated in ordinary broth under aërobic or anaërobic conditions. Some multiplication occurred in Martin's peptone broth to which blood-serum was added. After two to three days at 37° C. the clear broth became slightly opalescent. Microscopic examination did not reveal any definite increase of micro-organisms because the broth contained so many other particles. If a few drops of an alcoholic solution of eosin-blue was dropped in the broth the dye was decomposed as the virus multiplied and the eosin deposited as a ring along the walls of the tube, as shown by Marino.[4] An acidity was produced in sugar solution, up to 1 per cent. in glucose, levulose, and maltose. The acid was chiefly acetic acid. Acid was not produced in galactose, saccharose, lactose, or glycerin. Starch was not converted to sugar.

Growth was effected also on serum-peptone-agar, made from the same kind of broth as was utilized for fluid cultures. After four to five days colonies were visible under low magnification by direct light. Microscopically they were made up of fine refractile bodies which stained only partially. If the colonies were removed "in toto" from the agar and subjected to the action of dyes the bodies could be stained by ordinary anilin stains, gentian-violet, fuchsin, methylene-blue, and thionin, also by hematoxylin and hemin. They were Gram-negative. The larger elements stained badly and were regarded as involution forms. The organisms were apparently encapsulated. Growth occurred only above 30° C.

The virulence of the organisms was retained six to eight days in cultures. The infectious material could be preserved in glass ampules for a year at low temperatures, 6° to 12° C. It was not destroyed by strong glycerin, ½ of 1 per cent. carbolic acid. Destroyed at 58° C.

The organisms pass through Berkefeld filters and through Chamberland L2. The material containing them must first be diluted so that it has less than 15 per cent. of serum.

Bordet[5] obtained a sparse growth of the organisms of pleuropneumonia on blood agar medium made with glycerin potato extract. Microscopically these cultures showed fine threads, bent or twisted, S forms and spirals, together with round bodies with pale centers. Similar structures were found in fluid media containing 2 parts of peptone broth and 1 part serum or blood. The morphology was best brought out by Giemsa. Here Y forms occurred showing true branching. Bordet believes that the etiological agent of peripneumonia belongs to the vibrios.

The disease can be produced in cattle by intracutaneous and subcutaneous inoculation which gives rise to extreme edema with fatal outcome. At autopsy this edema is found to have passed through the muscles even into the pleura. Intrapleural inoculation causes extensive inflammation of the pleura and hepatization of the lungs. The peritoneum may also be involved. Fatal infection is also caused by intraperitoneal and intracranial injection of the virus. No infection from feeding, intravenous inoculation, or direct injection into the lungs. Sheep and goats are not susceptible (Dujardin-Beaumetz[6]).

Active immunity can be produced in cattle by subcutaneous injection at the end of the tail, both by infectious material and by cultures. The serum of such animals has some slight protective properties, does not agglutinate or dissolve the organism. After long treatment the serum of horses has agglutinating and precipitating properties, but no curative value.

1. Willems, L.: Mémoire sur la péripneumonie épizoötic du gros bétail, Bruxelles. 1852.
2. Pasteur: Rev. Vét., 1883, p. 64.
3. Nocard, Roux, Borrel, Salimbeniet, and Dujardin-Beaumetz: Ann. de l'Inst. Pasteur, 1898, 240–262.
4. Marino, F.: Ann. de l'Inst. Pasteur, 1905, xix, 816–820.
5. Bordet, J.: Ann. d. l'Inst. Pasteur, 1910, xxiv, 161–167 1910, p. 161.
6. Dujardin-Beaumetz, E.: *In:* Handb. der path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913. viii, 943–970.

## CATTLE-PEST

Cattle-pest or Rinderpest is a wide-spread epidemic disease in Europe, Asia, and Africa, known also as Viehpest, Hornviehseuche, Rindervieh-staupe, and Grossgalle. It is an acute infection with special lesions in the digestive tract. After an incubation period of three to six days, high fever (up to 40° to 41° C.), purulent conjunctivitis, and ulcers of the mouth and lips develop. These are accompanied by a bloody diarrhea. Death occurs after four to five days from general collapse. At autopsy the intestinal tract shows hyperemia and ulceration, there are pericardial effusions and hydrops in the brain cavities. Cases from different parts of the world exhibit somewhat different lesions. Thus the South African cattle-pest has more extensive pathological lesions in the intestines than the European variety.

The etiological agent in cattle pest is unknown and no specific organisms have been found in the blood or organs. A contagious material is present in the secretions of the infected animal, in the nasal mucosa, in the intestinal contents, regularly in the blood. Koch[1] has shown that the subcutaneous injection of the blood produces the disease in sensitive animals, with typical symptoms leading to fatal results. Theiler[2] has found that intravenous injection leads more surely to a fatal infection.

The disease is produced easily by the introduction of virulent material into the nasal passages. Small quantities of virulent blood (1/500 c.c.) suffice here to produce a fatal infection just like the natural disease, with an incubation period of three to six days, high fever, and death in nine to twelve days.

The infectious material has little resistance. It soon becomes inactive at room temperature and in the ice-box (in three to thirty-two days). At 36° to 40° C. it loses its activity in two days (Theiler). Dried blood loses it in four days (Koch). The virus is destroyed by distilled water in four to five days, glycerin in eight days, bile in two hours, 2 per cent. carbolic acid and 1:1000 bichlorid of mercury in a short time. It resists putrefaction and the addition of ½ per cent. carbolic acid to blood containing it. It is destroyed at 58° to 60° C. The virulence may be retained two to three months on hay kept in the dark.

Cattle-pest is transmissible regularly to cattle, but certain breeds are more resistant than others. Cattle from the steppes (Steppenrinder) are more resistant than ordinary cattle (Nicolle and Adil-Bey[3]), while animals from high mountains (Gebirgsrinder) are exceedingly sensitive. Ruediger[4] has shown analogous facts among the breeds of cattle in the Philippines. This difference in susceptibility may be related to the distribution of the disease and the resistance be an acquired immunity.

Sheep and goats have a high grade of natural resistance, but may be infected experimentally. Birds, dogs, cats, donkeys, rabbits, guinea-pigs, rats, and mice are not susceptible. Swine, camels, antelopes, and buffaloes develop the spontaneous disease somewhat infrequently. The infection is not transmissible to man.

Animals which have recovered from cattle-pest have a lifelong immunity. Koch has produced active immunity for prophylactic purposes by using the bile of infected animals. Kolle[5] thinks that the bile contains the fully virulent infectious agent and other materials which cause a localization of the virus when injected. In consequence the infection does not spread and an immunity develops. The immunity produced by bile may be increased by the subsequent injection of virulent blood after ten to thirty days (Kohlstock[6]).

Passive immunity can be brought about by injecting the blood of recovered animals or of those actively immunized by large doses of virulent blood. Combined active and passive immunization has been carried out by Bordet and Danysz (French method). The animals are first given large quantities of defibrinated blood from actively immunized animals and then have the virus introduced into the nasal mucosa. Kolle and Turner[7] have employed virus and immune serum at the same time (the simultaneous method), with favorable results.

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2. Theiler, A.: Schweitz Arch. f. Tierheilk., 1897, xxxix, 49–62; 193–213.
3. Nicolle, M., and Adil-Bey: Ann. de l'Inst. Pasteur, 1899, xiii, 319–343. *Ibid.*, 1901, xv, 715–733. *Ibid.*, 1902, xvi, 56–64, 291–293.
4. Ruediger, E. H.: Philippine J. Sc., 1908, iii, 165–181. *Ibid.*, 1909, iv, 425–452.
5. Kolle, W.: Ztschr. f. Hyg., 1899, xxx, 33–46.
6. Kohlstock: [Abstr.] Deutsche med. Wchnschr., 1898, xxiv, 724. [Abstr.] Centralbl. f. Bakteriol. [etc.], 1897, xxii, 787–791.
7. Kolle, W., and Turner, G.: Deutsche med. Wchnschr., 1897, xxiii, 793–795, 818–821.
*See also:* Sobernheim's Rinderpest in Kolle and Wassermann. 2. Aufl. 1913, viii, 971–992.

## STOMATITIS PAPULOSA OF CATTLE

This is a benign infectious disease of cattle with a vesicular eruption in the mouth. It can be experimentally transmitted by the blood, and the virus has been filtered through Chamberland candles by Ostertag and Bugge. Little is known about the properties of the virus, which has not been cultivated. One attack may confer immunity, but artificial immunity has not been produced. The mechanism of natural transmission is not known.

Ostertag and Bugge: Ztschr. f. Infektskr. u. Hyg. der Haustiere, 1906.

## FOOT-AND-MOUTH DISEASE

Foot-and-mouth disease (Maul und Klauenseuche), or aphthous fever, is widely prevalent in many parts of the world, especially Central Europe, India, Japan, and Sumatra. It has occasioned severe epidemics in North America and South America. The infection occurs naturally in beef, swine, sheep, goats, camels, horses, dogs, cats, stags, and does. It is characterized by aphthous ulcers on the mucous membranes of the mouth and on the feet, and on the udder in cattle.

Foot-and-mouth disease is transmissible to man, where the lesions occur chiefly on the lips, tongue, and mucosa of the cheeks. It may be transmitted by milk or whey (Dieckerhoff), by cheese or butter (Schneider, Frick, Fröhlich), and by direct contact (Hutyra-Marek[1]). In one human case

Bertarelli carried the infection back to calves. A human case has recently been described by Clough.[2]

The disease is not transmissible to rabbits, rats, mice, field-mice, hens, or pigeons.

The etiological agent is present in the lymph of fresh vesicles in which no organisms can be demonstrated as first shown by Loeffler and Frosch.[3] After filtration through Berkefeld filters which hold back ordinary small bacteria like *Bacillus fluorescens liquefaciens*, the lymph produces the disease in susceptible animals. Subsequently Loeffler and Frosch found that the virus multiplies in artificial media. The disease may be produced experimentally by intravenous and intramuscular injections.

The virus of aphthous fever is also found in the blood, but in very small quantities. After the outbreak of the disease it is everywhere present in the local lesions, especially in the lymph. It is very resistant to the action of disinfectants, but is destroyed almost immediately at 80° C., in five minutes at 60° C., in twelve hours at 50° C. In lymph diluted with salt solution and kept in the dark the virus remains active for a long time, occasionally five months.

The portal of entry of the natural disease is apparently the respiratory and digestive system and the injured skin. The milk of cattle contains the infectious agent and serves as the vehicle of transmission to man. It is doubtful whether the disease can be conveyed to man through the integument.

The causative organism of aphthous fever is not known. Frosch has described small bodies in the lymph 0.1 $\mu$ in length. V. Betigh[4] has found bodies 0.25 to 0.3 $\mu$ in size in the same material. Pfeiffer[5] has maintained the virus alive some time and found the v. Betigh bodies in it.

After an attack of the disease an immunity develops which may last several years. Casper[6] believes that successful methods of protective inoculation against the disease in cattle have not yet been devised.

Mezincescu, Baroni, and Colinescu[7] have recently filtered the virus through Chamberland and Berkefeld filters, but were unable to cultivate it in collodion sacs in the peritoneal cavity of young pigs or young chickens. The virus was attenuated by passage through rabbits by intraocular injection. The disease was also produced in cattle by the same method. The active virus was found in the serous liquid produced by burns in infected animals. After filtration through Berkefeld filters the virus was infectious for apes, *Macacus rhesus* and *Macacus pithecus*, producing an eruption on the mucosa and skin.

1. Hutyra, F., and Marek, J.: Spezielle Pathologie und Therapie der Haustiere. Jena, 6. Aufl. 1922, i, 383.
2. Clough, P. W.: Johns Hopkins Hosp. Bull., 1915, xxvi, 351–354.
3. Loeffler and Frosch: Deutsche med. Wchnschr., 1897, xxvii, 617. *Ibid.*, 1898, xxiv, 80–83, 97–100. Centralbl. f. Bakteriol. [etc.], 1. Abt., 1898, xxiii, 371–391.
4. v. Betigh, L.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1911, lx, Orig., 86–90.
5. Pfeiffer, W.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1924, xcii, Orig., 574–579.
6. Casper, M.: *In:* Handb. der path. Mikroorg. (Kolle and Wassermann). 2. Aufl. 1913, vi, 214–223.
7. Mezincescu, D., Baroni, V., and Calinescu, I.: Ann. de l'Inst. Pasteur, 1923, xxxvii, 1057–1063.

*See also* Lipschütz in Kolle and Wassermann, 1913, viii, 345–426.

## PERNICIOUS ANEMIA OF HORSES

Pernicious or infectious anemia of horses, also called swamp fever, is a disease which occurs in wet districts in Germany, France, and North America and is accompanied by a high mortality. It appears as an acute or chronic

septicemia with severe anemia and cachexia as a result and is usually fatal. It is not found in other species.

Horse anemia can be produced experimentally in normal animals by the injection of blood or blood-serum from an infected horse. The virus has been filtered by Carrée and Vallée[1] through a Berkefeld V and a Chamberland B and F. After the last method of filtration the incubation period is longer than after the first. These results were confirmed by Ostertag and Marek[2] and by Schlathölter,[3] who found that the incubation period in artificial infection can last up to three months. The virus is likewise present in the dejecta and in the urine. It stays alive a long time at room temperature, resists drying and putrefaction, is destroyed at 56° C. in one hour and by freezing at 0° C. in seven weeks. The infectious agent has not been cultivated. Ballah has described cell inclusions in the liver, but they are not specific.

Natural infection apparently occurs through the food and water contaminated by the urine. After recovery horses continue to give off the virus in the urine and are thus dangerous to well animals. There is no evidence of direct transmission from animal to animal, and transfer of the virus by flies or other insects has not been demonstrated (Kolle and Hetsch).

Kolle and Hetsch[4] report that there is no satisfactory evidence that pernicious anemia of horses is the same as South African horse sickness.

1. Carrée and Vallée: Compt. rend. Acad. d. sc., 1904, cxxxix, 331–333; 1239–1241.
2. Ostertag and Marek: Ztschr. f. Infektionskr. u. Hyg. d. Haustier. 1907.
3. Schlathölter: Ueber die perniziöse Anämia der Pferde. Inaug. Dissert. 1910.
4. Kolle, W., and Hetsch, H.: Die experimentelle Bakteriologie und die Infektionskrankheiten mit besonder Berücksichtigung der Immunitätslehre. 5. Auff. Berl., 1919, ii, 1209.

## SOUTH AFRICAN HORSE SICKNESS

South African horse sickness (Pferdesterbe, Pferdepest) is an infectious febrile disease of horses and mules which is wide-spread in South Africa and accompanied by a high mortality. It attacks animals at large more than those in stables. It is characterized by fever, subcutaneous edema about the eyes and in the tissues of the neck, and hemorrhages. Death occurs from pulmonary edema.

The disease can be produced experimentally in horses and mules by injecting the blood of sick animals subcutaneously, intravenously, and intrapleurally. The virus can be passed through Berkefeld and Chamberland B and F filters (Macfadyen[1]). It resists drying at 37° C. and may be preserved a long time, two years and four months (Nocard). It is not injured by rabbit bile, or 5 to 10 per cent. sodium taurocholate, 10 per cent. saponin, or 8 per cent. carbolic acid. It is not destroyed at 45° C., but loses its vitality at 50° C. When mixed with 5 to 10 per cent. lecithin the virus is not infectious, regaining its infectivity after separation from this substance. The virus does not pass collodion sacs or 0.1 per cent. agar. It does not survive in water.

Small quantities of blood (0.0001 c.c.) produce infection by inoculation, and large quantities, 150 to 200 c.c., by feeding. The virus is in the blood during the entire febrile period, in the conjunctival and bronchial secretions, in the edematous fluid in the lungs, but has not been found in the dejecta.

The infectious agent has not been cultivated. Kuhn[2] has found cell inclusions in the kidney epithelium like those of trachoma. They may be so large as to press the nucleus to one side. They resemble the inclusions in fowl-pox (Chlamydozoa).

Dogs may become infected by eating the meat of infected horses.

Horse sickness is carried by biting insects, as proved experimentally for Anopheles and Stegomyia mosquitoes by Theiler,[3] and for *Stomoxys calcitrans* by Kuhn and Schuberg. The disease disappears spontaneously eight days after frost begins. Ticks may harbor the virus, but transmission through their agency has not been proved.

After recovery horses have a certain degree of immunity and animals can be experimentally immunized by the virus, but not against large doses. Horses which have recovered from the natural disease may have their immunity raised by increasing doses of virulent blood. If their serum be mixed with small quantities of virulent blood and administered to normal animals (simultaneous method) an immunity is set up which lasts about a year (Koch, Theiler, and Rickmann).

1. Macfadyen: J. Comp. Path. and Therap., 1900, 1901.
2. Kuhn, P.: Centralbl. f. Bakteriol. [etc.], 1911, l, Ref. Beiheft, 31–33.
3. Theiler, A.: Annual Rep. of the Gov. Veter. Bacteriol. for 1903–1904, Pretoria, 1905, 131–172. [Abstr.] Bull. de l'Inst. Pasteur., 1905, iii, 802.

## HORSE-POX

Horse-pox, variola equina, or sore heels, was described by Jenner as a vesiculopustular eruption of the angles of the fetlocks. The disease known as *stomatitis pustulosa contagiosa* is also regarded by English veterinarians as horse-pox. The condition observed by Jenner was epidemic and thought by him to be the source of cowpox. It is very rare at the present time.

In some cases called horse-pox Bormans has found structures in the lymph like the "elementary bodies" of vaccinia. Loy has reported the artificial transfer of the infection to man in seven cases, while Hertwig and Pinkau have observed the natural transfer to man.

See Hytyra-Marek: Spezielle Pathologie und Therapie der Haustiere, Sechste Aufl., 1922, i, p. 380.

## HORSE DISTEMPER

A disease known as horse distemper, horse typhoid, pink eye, and Pferdestaupe has been known among veterinarians for years. Its contagious character was established by Clark[1] in 1894. It has been investigated especially by Jensen,[2] Reeks,[3] Grimme,[4] and Spillman.[5] Bemelmans[6] finds the virus of the disease in the blood and has filtered it through Berkefeld and Chamberland F. It is also present in the sperm of stallions and can likewise be filtered from this medium.

The virus can be preserved for a month at room temperature, but not for three months. It is retained in a virulent condition in the seminal vesicles of stallions for long periods, even three years. In the artificial disease the period of incubation is three to five days and the malady lasts ten to twelve days.

The virus has not been cultivated and belongs in the group of ultramicroscopic infectious agents.

After the natural and experimental disease a high-grade immunity is established so that the animals can resist the injection of massive doses of virulent blood (10 c.c.).

1. Clark, J.: J. Comp. Path. and Therap., 1894, v.
2. Jensen: Deutsch. Ztschr. f. Tiermed., 1894.
3. Reeks: J. Comp. Path. and Therap., 1902.
4. Grimme, A.: Deutsche tierärztl. Wchnschr., 1903, xi, 109–111.
5. Spillman: Trans. 9. International Congress of Veterinary Medicine, Hague, September, 1909.
6. Bemelmans, E.: Centralbl. f. Bakteriol. [etc.], 1913, lxiii, Orig., 8–28.

## BORNA'S DISEASE

Borna's disease, enzoötic cerebrospinal meningitis of horses, is an infectious disease which begins in the early months of the year, reaches its height in the spring, recedes in summer, and disappears in the autumn. It occurs chiefly in workhorses on farms. Joest[1] describes the disease as an acute disseminative, infiltrative, non-purulent encephalitis, accompanied by a meningitis. It has not been transmitted definitely to other horses, and the cerebrospinal fluid and brain substance are not injurious to mice, guinea-pigs, or rabbits.

Johne and Ostertag have found diplostreptococci in Borna's disease, but these have not been proved to be the etiological agent. Joest believes that the etiological agent cannot be pyogenic in character because of the type of the lesions. He has described nuclear inclusions in the large ganglion cells of the brain, especially in Ammon's horn, demonstrated by Lentz's Stain A for Negri bodies.[2] These inclusions stain light red and are easily differentiated from the blue nuclear cytoplasm and the violet nucleolus. They are also stained by Heidenhain's iron-alum hematoxylin and by Giemsa. They are round or diplococcus-like in form, vary in number from one to six, and in size from just visible bodies to bodies the size of red blood-corpuscles. They are homogeneous, without structure, surrounded by a light colorless zone. They consist of plastin.

Joest's nuclear inclusions occur only in the nuclei of the ganglion cells and are regarded as just as specific for Borna's disease as the Negri bodies for rabies. They may be reaction products of the ganglion cells against the invasion of an organized parasitic agent belonging to the Chlamydozoa of v. Prowazek.

1. Joest, E., and Degen, K.: Ztschr. f. Infektionskr. . . . d. Haustiere, 1909, vi, 348–356. *Ibid.*, 1911, ix, 1–98.
   Joest, E.: *In:* Handb. der path. Mikroorg. (Kolle and Wassermann). 1913, vi, 251–268.
2. Lentz, O.: Centralbl. f. Bakteriol. [etc.], 1907, xliv, 374–378.

## HOG-CHOLERA

Hog-cholera (Schweinepest) or swine fever is a highly contagious disease of swine not transmissible to other species, characterized by peculiar ulcers called buttons in the mouth and along the entire length of the alimentary canal. The mortality is high, the animals dying of a bloody diarrhea and emaciation. The disease is wide-spread in Europe and America.

Hog-cholera has long been known to be contagious, passing rapidly from infected to normal animals. At first regarded as due to organisms belonging to the paratyphoid group, it was produced experimentally by de Schweinitz and Dorset[1] by the bacteria-free blood and blood-serum. Subsequently the virus of the disease was shown by Dorset, Bolton, and McBryde[2] to be filtrable, passing through a Chamberland B and F. These observations were confirmed by McClintock, Boxmeyer, and Siffer,[3] by von Ostertag and Stodie, by Poels,[4] by Uhlenhuth, Hübener, Xylander and Bohtz,[5] and by Haendel, Gildemeister, and Schirn. The virus is uniformly present in the blood, small quantities, 2, 1, 0.05, and 0.01 c.c., producing the disease by subcutaneous injection.

The virus of hog-cholera is also present in the purulent urine and in all the organs. A small quantity of the eye secretions produces the disease when dropped in the normal eyes of shoats (Uhlenhuth), and this infection may be carried on from eye to eye. Occasionally the disease can be produced by feeding.

The virus survives fairly long outside the body, fourteen days in the urine. It may be preserved in liquids at room temperature ten to fourteen weeks. When moist it resists a temperature of 45° to 46° C. twenty-four hours, but is inactive after exposure to 46° C. for forty-eight hours, 55° to 60° C. for twenty-four hours, 60° C. for ten hours, 58° C. for two hours, and 70° C. for one hour. Virulent blood after drying at 37° C. resists a temperature of 65° C. for two hours, but is destroyed in one hour at 72° C., 76° C., and 100° C., in one-half hour at 150° C. It survives drying several days at 37° C. In serum the virus can be preserved on ice four to six months. It resists direct sunlight five to nine hours, but is destroyed by putrefaction. In the urine the virus is destroyed by sublimate in strengths of 2 to 3 per 1000, while in the blood it may resist this strength. It is killed slowly by 0.5 per cent. carbolic and 5 per cent. glycerin (eight days). It is destroyed by $2\frac{1}{2}$ per cent. formalin in fifteen days in the ice-box, by 5 per cent. in two hours at room temperature. It is not destroyed by chloroform or sodium taurocholate.

The etiological agent of hog-cholera has not been cultivated and organisms are not visible in the disease by ordinary microscopes or by dark field illumination. Cell inclusions have been described by Uhlenhuth and Böing[6] in the conjunctiva five to six days after infection. They can be stained by Giemsa and are then violet in color. The size varies, but the inclusions are usually larger than trachoma bodies. There is some question about the etiological rôle of these inclusions, since they have been found in normal swine in about 3 per cent. of the examinations.

Swine have little natural immunity to hog-cholera, but complete immunity follows an attack of the disease or its artificial production. Animals recovered from the experimental disease are resistant to natural and artificial infection. The serum of actively immunized animals induces passive immunity as first shown by Preisz.[7] Several methods of protective inoculation have been practiced. The animals may be given several doses of virulent blood, immune serum followed by virulent blood, or immune blood and virus at the same time.

1. de Schweinitz and Dorset: Rep. Bureau Animal Indust., Cir. 41, 1903.
2. Dorset, M., Bolton, B. M., and McBryde, C. N.: Rep. Bureau Animal Indust., 1904, xxi, 138–158.
3. McClintock, C. T., Boxmeyer, C. H., and Siffer, J. J.: J. Infect. Dis., 1905, ii, 351–374.
4. Poels: De Varkenozickten in Nederland's Grovenhage. 1905.
5. Uhlenhuth, Hübener, Xylander, and Bohtz: Arb. a. d. K. Gsndhtsamte, 1908, xxvii, 425–671.
   Uhlenhuth and Hübener: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1909, xlii, Ref., Beihft., 127–138.
6. Uhlenhuth and Böing: Berl. klin. Wchnschr., 1910, xlvii, 1514–1515.
7. Preisz, H.: Ztschr. f. Thiermed., 1898, ii, 1–66.

## HEART-WATER DISEASE

Heart-water is a specific infectious disease affecting sheep, goats, and cattle in South Africa, the most characteristic lesion of which is hydropericardium. During life the affected animals show a fever of 105° to 108° F., the temperature dropping to subnormal before death; nervous symptoms, muscular twitching, tetanic spasms, squinting, and excessive salivation. The mortality is high, about 50 per cent. Lounsbury[1] has transmitted the disease to sheep, goats, and cattle. The virus is in the blood and Edington[2] has produced the disease in susceptible cattle by the inoculation with it. Theiler and Robertson[3] find that the virus does not pass through Berkefeld or Chamberland filters.

Heart-water is transmitted by the bont tick, *Amblyomma hebræum* (Koch), as first shown by Lounsbury and by Theiler and Robertson.[3] Lounsbury demonstrated that normally the tick does not carry the virus, but gets it by feeding on diseased animals. It carries it through the moulting period from larva to nymph or from nymph to adult. The larva after receiving the virus and having fed as nymph on an insusceptible animal, can transmit it in the adult stage to the next host if this be susceptible. The period of incubation is fourteen days after the bite of the tick, less after blood inoculation.

Cowdry[4] has recently transmitted heart-water to sheep, goats, and cattle, and has found peculiar bodies in the epithelial cells of the capillaries of the renal glomeruli, in the superficial gray matter of the cerebral cortex, in the cerebellar cortex, and in various other regions, including lymph-glands, spleen, corpus luteum, suprarenals, ovaries, salivary glands, and pancreas. They were not found in the liver or lungs. These bodies occurred in clumps, up to several hundred, appeared usually as cocci 0.2 to 0.5 $\mu$ in diameter, occasionally as diplococci. They stained deep clear blue by Giemsa and were easily demonstrated by Loeffler's methylene-blue, fuchsin, and iron hematoxylin. They appeared light red or slightly greenish by Unna-Pappenheim's methyl-green-pyronin mixture. They were Gram-negative.

The bodies found by Cowdry are regarded by him as micro-organisms belonging to the Rickettsias and have been named *Rickettsia ruminantium*.

The same structures have subsequently been found by Cowdry[5] in infected ticks where they are abundant in the intestinal epithelial cells of both larvæ and nymphæ. They look like minute cocci and usually measure 0.2 to 0.3 $\mu$ in diameter. Diplococci measuring 0.2 by 0.8 $\mu$ and bacillary forms 0.2 to 0.3 $\mu$ by 0.4 to 0.5 $\mu$ also occur. They are present only in infected *Amblyomma hebræum*, not in other ticks, and are limited to the epithelial cells of the intestine and to the lumen of the gut. If ticks be fed with *Rickettsia ruminantium* they can produce heart-water in susceptible animals. Ticks without Rickettsias cannot convey the infection.

Cowdry states that *Rickettsia ruminantium* resembles the type species *Rickettsia Prowazeki* more than the Rickettsias of Rocky Mountain spotted fever do, particularly as regards their position in the epithelial cells lining the intestine and in the absence of intranuclear forms. They have not been cultivated.

1. Lounsbury, C. P.: Agric. J. Cape of Good Hope, 1902, xx, 29; xxi, 165.
2. Edington, A.: Agric. J. Cape of Good Hope, 1904. J. Comp. Path. and Therap., 1904, xvii, 141.
3. Theiler and Robertson: Ann. Rep. Gov. Vet. Bact. for South Africa, 1903–04, 114.
4. Cowdry, E. V.: J. Exper. M., 1925, xlii, 231–252.
5. Cowdry, E. V.: J. Exper. M., 1925, xlii, 253–274.

## NAIROBI SLEEPING SICKNESS

This is a plague of sheep and goats in East Africa, the virus of which has been shown by Montgomery to pass Berkefeld and Chamberland filters. The disease is transmitted by the blood, possibly by the feces, not by co-habitation. Immunity follows an attack, whether lasting or not has not yet been established. The local sheep are very susceptible and have a high mortality.

Montgomery, R. E.: Annual Report of the Veter. Path. for 1910–11, East African Protectorate, Ref. Arch. f. Schiffs-u. Tropenhyg., 1913, xvii, 140.

### CONTAGIOUS PUSTULOUS STOMATITIS OF SHEEP

Described by Aynaud[1] as an infectious disease of sheep, especially lambs, characterized by the presence of thick crusts on the lips and face and in the mouth. These are button-like and resemble chancres, so that the disease is sometimes called "chancre du mouton." The mortality is high, especially when the mouth is affected with stomatitis. Aynaud believes that the mouth lesions and the face lesions are due to the same infectious material. Goats are also affected. The disease occurs in epidemics and is highly contagious. In fatal cases bronchopneumonia and pulmonary abscesses are found due to organisms of the hemorrhagic septicemia group (Pasteurella).

The material from the lesions on the lips and mouth produces similar lesions on the skin. There is no general reaction, but the neighboring lymphatic glands enlarge. Lesions can be produced on the vulva also, but not on the cornea. Intravenous inoculation and ingestion have no effect.

The disease is transmissible experimentally to goats, not to rabbits, guinea-pigs, young pigs, fowls, pigeons, or frogs. Man is apparently resistant.

The virus in the pustules is very powerful. A dilution of 1 : 10,000 gives positive results regularly, of 1 : 100,000 occasionally. The virus is not found in the blood or organs. If the lymph be diluted the virus can be filtered slowly through a Berkefeld V, Chamberland L1 and L1 bis. It is not destroyed by putrefaction and survives twenty-four hours at 37° C. when mixed with other bacteria. Destroyed at 57° C. in forty-five minutes, at 61° C. in ten minutes, at 74° C. in five minutes, at 86° C. in one minute. The dried virus is killed in sixty minutes at 80° C., in ten minutes at 100° C. Destroyed easily by light and by disinfectants of medium strength.

Immunity develops after recovery from the natural and experimental disease. Local immunity may be produced in the cornea, but the skin immunity does not extend to this region. Vaccination by intravenous inoculation produces a general immunity of which the cornea partakes and the disease can be prevented by vaccination. Vaccination by the digestive tract gives no protection.

Blanc, Mélanidi, and Caminopetros[2] have reported an eruptive disease of goats in Greece, not transmissible to guinea-pigs or rabbits. This may be the same disease.

1. Aynaud: Ann. de l'Inst. Pasteur, 1923, xxxvii, 498–527.
2. Blanc, G., Mélanidi, C., and Caminopetros, J.: Ann. de l'Inst. Pasteur, 1922, xxxvi, 614–618.

### AGALACTIA OF SHEEP AND GOATS

This is an infectious disease of goats and ewes in Italy, characterized by local lesions in the eyes, joints, and udders. The disease usually results in permanent loss of milk and blindness. It is transmitted by contact and can be produced experimentally by a virus which has been filtered through Berkefeld candles by Celli and De Blasi. The virus is also in the milk. Not cultivated.

### SHEEP-POX

Sheep-pox (clavelée) is a widely prevalent disease in certain parts of Europe, Asia, and Africa, with a generalized eruption of papules, vesicles, and pustules. It belongs in the general group of diseases known as pocks or pox. It is contagious from animal to animal, has a low mortality, and leaves behind a complete immunity after recovery.

Kolle and Hetsch[1] regard sheep-pox as like cowpox, but a different disease

and not the same as the pox produced in sheep by cowpox virus. It is transmissible to sheep, but not to other animals except perhaps to goats, but Nocard was unable to transmit sheep-pox in France to these animals. It is not conveyed to man. The pustules occur on the skin and mucous membranes, on the mucosa of the intestines and air passages. The virus of the disease is found in these pustules and in the juice of the internal organs. It is present in the circulating blood for only a short time, just before the general eruption.

Borrel[2] has filtered the virus of sheep-pox through a Chamberland F4 and F6. It resists drying and retains its virulence two years if kept in a cool place. It withstands freezing for two months, resists 50 per cent. glycerin twelve days, also 3 per cent. boric acid, 2 per cent. salicylic acid, and 10 per cent. chlorid of lime. It is destroyed at 48° C. by putrefaction, by 1.25 per cent. carbolic acid, 2.5 per cent. sulphuric acid, by 1 : 10,000 iodin solution. It has not been cultivated.

Borrel[2] has described large cells in the purulent contents of the pustules, 40 to 50 $\mu$ in diameter, containing characteristic cell inclusions near the nuclei. These are regarded as reaction products of the cells against the virus, similar to reaction products against other dermotrophic viruses. Borrel and Paschen have also found large numbers of small bodies which can be stained by Giemsa or Loeffler. These correspond to the elementary corpuscles of the Strongyloplasmata of v. Prowazek. According to Lipschütz they may be the virus.

The serum of recovered animals contains immune substances, and sheep may be actively immunized, their serum giving a partial passive immunity. Vaccination can be carried out by the contents of the pustules.

Kolle and Hetsch believe that infection may follow inhalation of the virus.

1. Kolle, W., and Hetsch, H.: Die exper. Bakteriol., u. die Infektskr. mit besondere Berucksichtsetz der. Immunitätslebre. 5. Aufl. 1919, ii, 1211.
2. Borrel, A.: Ann. de l'Inst. Pasteur, 1903, xvii, 123; 732.

## CATARRHAL FEVER OF SHEEP

Catarrhal fever of sheep or blue tongue occurs in South Africa and is marked by congestion and edema of the mouth and tongue and about the coronets of the feet. The mortality varies from 5 to 30 per cent. The disease cannot be transmitted to other species, and the mechanism of its transmission to animals of the same species has not been worked out. It may take place by contact or through an intermediate host.

Blue tongue can be produced experimentally by the blood. The virus can be filtered through Berkefeld candles, as shown by Robertson and Theiler and by Spreull. It resists 25 per cent. glycerin plus 0.25 per cent. carbolic acid two years and 50 per cent. glycerin ten days. In the blood it survives four hundred and sixty-seven days. It resists putrefaction, carbolic acid of less than $2\frac{1}{4}$, or 2 per cent. boric acid seven days, $\frac{1}{4}$ per cent. formalin twenty-five days. Not cultivated.

After recovery animals are immune, and a protective serum can be produced by the immunization of sheep.

Spreull: J. Comp. Path. and Therap., 1905, xviii.

## FOWL PEST

Fowl pest (Hühner-pest) occurs in fowls, pheasants, turkeys, sparrows, owls, parrots, and blackbirds, rarely in young geese and young doves. Pigeons, old geese, and ducks are resistant to the natural disease, which is

not transmissible to mammals. The infection is marked by sudden onset, bloody diarrhea and high mortality, resembling fowl-cholera. Under natural conditions it seems to be transmitted by food contaminated by feces and nasal secretions.

The infectious agent of fowl pest is a virus which is present in the blood-stream, which has been shown to be filtrable by Lode and Gruber,[1] by Centanni,[2] and Ostertag and Wolffhügel.[3] It usually passes Berkefeld filters and Chamberland F, but not K. It resists glycerin two hundred and seventy-five days. When dried in the spinal cord or liver it survives two hundred days. It is not destroyed at 60° C. in one-half hour (Lode and Gruber), but is killed at 64° to 74° C. in the same time (Centanni). It survives sixteen days at 37° C., thirty-three days in infected fowls. It is destroyed by putrefaction, by 1 per cent. corrosive sublimate in thirty minutes, by 1 per cent. sulphuric acid in ten minutes. In brain tissue it is destroyed at 65° to 68° C. only in four hours.

The disease can be produced experimentally by the virus in the animals sensitive to natural infection, but the distribution of the virus varies considerably in different species in both natural and experimental cases. Thus in fowls the pest is a septicemia with the virus in the blood and all the organs, in the nasal mucosa, dejecta, bile (Lode and Gruber); it also passes into the egg (Centanni). In young geese also a septicemia appears, with the virus in the central nervous system after subcutaneous or intramuscular injection (Kleine). In old geese, which are resistant to the natural disease and to subcutaneous or muscular inoculation, the virus can be found in the spinal cord and in the liver after cerebral injection (Kraus and Schiffmann[4]). In old pigeons also, after cerebral injection, a septicemia develops with the virus in the central nervous system. After cerebral injection in rabbits, which are naturally resistant, the virus multiplies in the thoracic cord. It disappears from this area and from the site of injection in a few days. It disappears from the blood of rabbits in twenty-four to forty-eight hours (Kraus and Doerr[5]).

The virus is highly infectious, 4 c.c. of a 1 : 125,000,000 blood suspension carrying infection to hens (Maggiora and Valenti[6]). In the blood of geese it is less concentrated. Fowls injected with 1 c.c. of goose blood sometimes die, with 2 c.c. always. The infection can be transmitted to fowls by feeding, subcutaneous, and intramuscular inoculation.

The etiological agent of fowl pest has not been determined definitely. Von Prowazek[7] has described in the cerebrum, vermis, and medulla round or oval circumscribed bodies 1 to $1\frac{1}{2}$ $\mu$ in size, single or in clumps. These stain light yellow-rose by Giemsa and contain round or elongated bacteria-like bodies which are dark red in color. Occasionally the bodies look like dumb-bells and seem to have divided by simple fission. If the filtered infectious material be centrifugalized and stained by Loeffler, minute bodies $\frac{1}{4}$ to $\frac{1}{2}$ $\mu$ in size, points and double points, may be demonstrated. These are regarded as a second stage of the virus by v. Prowazek, against which reaction products are developed in the cells.

Marchoux[8] has brought the infectious agent of fowl pest to cultivation in a medium consisting of agar, 1 per cent. peptone, 2 per cent. sugar, and defibrinated fowl blood. After ten generations 2 c.c. of material taken at the junction of the agar and the blood from a three-day culture at 37° C. produced the disease in fowls, with death in two days. Here the virus must have multiplied. This has been confirmed by Landsteiner.[9]

Animals which have recovered from fowl pest are immune and their serum in large quantities (10 c.c.) destroys the virus (Ostertag and Wolff-

hügel). Geese can be immunized against the spinal cords of young geese which have been injected in the muscles and have the virus in the spinal cord, by the use of the dried spinal cords.

1. Lode, A., and Gruber, J.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1901, xxx, 593–604.
2. Centanni: Clinica Veterinaria, 1901, xxiv, 292.
3. Ostertag and Wolffhügel: Monatsh. f. prakt. Tierheilk., 1902, xiv, 49.
4. Kraus, R., and Schiffmann, J.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1907, xliii, Orig., 825–832.
5. Kraus, R., and Doerr, R.: Centralbl. f. Bakteriol. [etc.], 1908, xlvi, 709–715.
6. Maggiora, A., and Valenti, G. L.: Ztschr. f. Hyg., 1903, xlii, 185–243.
7. v. Prowazek, S.: München. med. Wchnschr., 1908, lv, 165–166.
8. Marchoux, M. E.: Compt. rend. Acad. d. sc., 1908, cxlvii, 357–359.
9. Landsteiner, K.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1906, xxxviii, Ref., 540–542.
*See also:* von Ostertag's Fowl Pest in Kolle and Wassermann. 1913, vii, 280–297.

## FOWL-POX

Fowl-pox or epithelioma contagiosa of fowls is a disease of the skin in fowls, turkeys, pigeons, and hawks, affecting all parts not covered by feathers, such as the comb, larynx, eyelids, angles of the bills, ear, under surface of the wings, and often the feet. It appears as nodules the size of poppy seeds, hemp seeds, or larger, grayish or grayish-yellow, with a peculiar glistening appearance like mother-of-pearl. When the nodules occur on the lids these structures are swollen and puffed out. The conjunctiva may show catarrhal inflammation and be buckled by the nodules. The entire eye may be covered by a mulberry-like growth, especially in pigeons.

In the interior of the nodules round or oval bodies are present in the degenerated epithelial cells and free in the tissue spaces, surrounded by clearly stained capsules. These are sometimes called molluscum bodies and are like the molluscum bodies in molluscum contagiosum in man. They are now regarded as cell degenerations. If the preparations be stained by Giemsa or Loeffler's flagella stain peculiar elements are to be found surrounding the molluscum bodies as first demonstrated by Borrel.[1] These fowl-pox bodies are round, single, double or in clumps, about $\frac{1}{4}$ $\mu$ in diameter, and Gram-negative. They lie in the hypertrophic rete cells near the nuclei.

Some difference of opinion has arisen as to the interpretation of the fowl-pox bodies. They have been grouped among the Strongyloplasmata by Lipschütz[2] and called elementary bodies. Sanfelici considers the red staining elements in the molluscum bodies as primarily of nuclear origin. At the present time the fowl-pox bodies are usually regarded as reaction products of the cells against the virus of the disease.

Fowl-pox is transmissible from bird to bird by inoculation of material from the lesions. The virus has been passed through Berkefeld filters by Marx and Sticker.[3] It is present in the blood nine to ten days after inoculation in the experimental disease, and in the internal organs, especially in fowls recovered from the infection. The incubation period is four to five days (Burnet). The mortality of the natural disease varies from 6 per cent. (Juliusberg) to 97 per cent. (Sanfelici).

The virus of fowl-pox is extremely resistant and retains its virulence a year when dried. It is not destroyed at 60° C. in three hours. In dried tumor masses in closed vacuum tubes it resists steam one hour and 5° C. for five weeks. It is not killed by drying and sunlight in two months. It survives in pure glycerin thirty days, is not injured by 0.1 per cent. carbolic, but is destroyed in 1 per cent. in one-half hour.

The virus of pigeon-pox is somewhat less resistant than that of fowl-pox.

It is destroyed at 60° C. in fifteen to thirty minutes and at 70° C. in five minutes. After passage through hens it loses its virulence for pigeons.

Fowl-pox is transmitted under natural conditions apparently through small injuries of the skin. Experimentally it can be produced by inoculation of the skin and by feeding material containing the virus (Burnet[4]).

One attack of the disease confers an immunity. Artificial immunization has been accomplished by Manteufel.[5]

The relationship of fowl-pox to other pox diseases is not entirely clear. Carnwath[6] regards the virus as identical with that of fowl diphtheria, but this is not accepted by v. Betigh and Bordet. Some authors regard it as the same as molluscum contagiosum, but no satisfactory evidence has been presented to show that fowl-pox is transmissible to man.

Fowl-pox apparently has no relation to smallpox or to cowpox. Loewenthal, Kadowaki, and Kondo[7] have recently shown that vaccinia may be transmitted to fowls and carried back to rabbits where it is still vaccinia with epithelioid growth and Guarnieri bodies. Fowl-pox can also be carried to rabbits, but shows no Guarnieri bodies. The two viruses also convey specific immunity. The cause of fowl-pox must, therefore, be different from that of vaccinia.

1. Borrel, A.: Compt. rend. Soc. de biol., 1904, ii, 642–643.
2. Lipschütz, B: Centralbl. f. Bakteriol. [etc.], 1908, xlvi, Orig., 609–622.
3. Marx, E., and Sticker, A.: Deutsche med. Wchschr., 1902, xxviii, 893–895. *Ibid.*, 1903, xxix, 79–80.
4. Burnet, E.: Ann. de l'Inst. Pasteur, 1906, xx, 742–765.
5. Manteufel: Arb. a. d. K. Gsndhtsamte, 1910, xxxiii, 305–312.
6. Carnwath, T.: Arb. a. d. K. Gsndhtsamte, 1908, xxvii, 388–402.
7. Loewenthal, W., Kadowaki, Y., and Kondo, S.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1925, xciv, Orig., 185–200.

## FOWL DIPHTHERIA

Fowl diphtheria is a peculiar disease of fowls characterized by an exudative diphtheritic inflammation of the mucous membranes. It causes death by edema of the lungs, serous exudate in the joints and serous cavities, and necrosis of the liver. Guinea-fowls, pigeons, and pheasants also are susceptible. It is apparently transmitted by contact and by ingestion of exudates and infected epithelial cells.

Fowl diphtheria is frequently accompanied by nodular growths in the skin and mucosa of the neck and head. These nodules may degenerate and cause blindness and inability to use the mouth. Therefore it is not clear whether the fowls have two diseases, fowl-pox and fowl diphtheria, or whether fowl-pox and fowl diphtheria are the same. Carnwath[1] regards them as identical.

Epithelial inclusions have been described by v. Prowazek as Chlamydozoa. Immunity develops after an attack of the natural or experimental disease. Passive immunity has not been demonstrated.

Bordet and Fally[2] do not accept the identity of fowl-pox and fowl diphtheria. They believe the virus of fowl diphtheria can be cultivated in glycerin potato agar to which defibrinated rabbit's blood is added. The inoculated areas of the medium turn dark and show microscopically minute non-motile rod-like bodies which are best stained by Giemsa. With these cultures the disease could be produced in the mucous membranes of the mouth in fowls. Growth also occurred as a granular deposit in peptone broth containing one-half volume of rabbit's serum fresh or heated to 58° C. one-half hour.

1. Carnwath, T.: Arb. a. d. K. Gsndhtsamte, 1908, xxvii, 388–402.
2. Bordet, J., and Fally, V.: Ann. de l'Inst. Pasteur, 1910, xxiv, 563–568.

### FOWL LEUKEMIA

In addition to the spontaneous leukemia of fowls studied by Moore,[1] which is an acute bacterial infection accompanied by a hyperleukocytosis, an infectious leukemia occurs which is due to a filtrable virus, as first shown by Ellermann and Bang.[2] This occurs in epidemics and is marked by leukemic blood, swelling of spleen and liver, and changes in the bone-marrow. Emulsions of the liver, spleen, and bone-marrow in 0.9 per cent. salt solution produce the disease in healthy fowls by intravenous and intraperitoneal inoculation. The experimental disease has an incubation period of one to two months and presents a striking similarity to spontaneous leukemia. The disease can be transmitted from fowl to fowl by the cell-free virus after filtration through Berkefelds. Intravenous inoculation is most certain, intraperitoneal injection is also successful, subcutaneous negative. The material loses its virulence in a few days even at low temperatures. The disease is not transmissible to other birds (pigeons, guinea-hens) or to rabbits.

Hirschfeld and Jacoby[3] have described inclusions in the leukocytes which stain reddish by Giemsa and red with methylene-green pyronin. They are non-motile and show no structure. Such inclusions may be reaction products against the infectious agent which belongs to the hemotrophic viruses of Lipschütz.

1. Moore: Infectious Leukemia in Fowls.
2. Ellermann, V., and Bang, O.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1908, xlvi, Orig., 4–5, 595–609. *Ibid.*, 1909, lxiii, 231–272.
3. Hirschfeld, H., and Jacoby, M.: Berl. klin. Wchnschr., 1909, xlvi, 159–160.

### BLACKBIRD SEPTICEMIA

A highly fatal disease in blackbirds, *Turdus merula* and *Turdus viscivorus*, described by Maggiori and Valenti as due to a virus which is found in the blood and organs and can be passed through Berkefeld filters. The infection can be transmitted by subcutaneous injection and feeding to blackbirds, falcons, hawks, and owls. Sparrows and pigeons are difficult to infect, hens and small laboratory animals refractory.

Maggiora, A., and Valenti, G. L.: Centralbl. f. Bakteriol. [etc.], 1. Abt., 1903, xxxiv, Orig., 326–335.

### ROUS' CHICKEN SARCOMA

A spindle-celled sarcoma of chickens, transmitted by a virus passing Berkefeld filters, but not Chamberland F, has been described by Rous. The virus resists 50 per cent. glycerin one month, freezing and thawing, drying seven months, heating to 50° to 53° C. It is destroyed at 55° C. in fifteen minutes, by 5 per cent. alcohol and 2 per cent. phenol in two hours. It is easily killed by saponin and by bile.

### NOVY'S RAT DISEASE

A disease of rats due to a filtrable virus has been described by Novy. The virus is in the blood, passes through the finest porcelain filters, and cannot be deposited by the most powerful centrifugation.

### GUINEA-PIG DISEASES

Two diseases have been described in guinea-pigs due to filtrable viruses. One was found in the Lister Institute by Petrie and O'Brien.[1] It had a high mortality, only 21 of 500 infected animals recovering. A filtrable

virus was present in the blood-serum which passed through Berkefeld filters and could not be cultivated. The animals also had an intestinal infection with organisms of the paratyphoid group. This disease may be identical with the guinea-pig pest or plague of de Gasperi and Sangiorgi,[2] also caused by a filtrable virus.

Roemer[3] has found a guinea-pig paralysis characterized by fever, paralysis of the bladder and hind legs, with emaciation. It lasts from three to twenty-one days. The pathological changes are somewhat like poliomyelitis in man. Guinea-pig paralysis is also caused by a virus which passes through the Berkefeld filters. It resists 50 per cent. glycerin ten days and has not been cultivated.

1. Petrie, G. F., and O'Brien, R. A.: J. Hygiene, 1910, x, 287–305.
2. de Gasperi, F., and Sangiorgi, G.: Centralbl. f. Bakteriol. [etc.], 1913, lxii, Orig., 257–266.
3. Roemer: Proceedings International Congress of Hygiene, Dresden, 1911.

## SANARELLI'S MYXOMATOUS DISEASE OF RABBITS

Sanarelli has reported an epidemic disease of rabbits in which the animals show severe blepharitis, with completely closed eyelids, and tumors in various parts of the body composed of myxomatous tissue, chiefly star-shaped cells. Death occurs in a few days. At autopsy the spleen and lymph-glands are swollen from new-formed myxomatous tissue. The disease is easily conveyed to rabbits by subcutaneous and intravenous inoculation, by mouth, or through the eyes. The virus is present in the blood and organs and cannot be separated by centrifugation. Moses has reported a filtration through Berkefeld, but not through Chamberland. The virus resists formol, glycerin, hydrogen peroxid, is destroyed by carbolic acid, sublimate solution, chloroform, saponin, and bile. Aragão regards the virus as visible and belonging to the Strongyloplasmata.

## MOSAIC DISEASE OF TOBACCO

The contagious character of Mosaic disease of tobacco was first demonstrated by Meyer,[1] who squeezed out the juice of diseased plants, inoculated healthy plants with it, and produced the disease. The virus was shown to be filtrable by Iwanowski[2] in 1892, and this is the first demonstration of organisms of this type, antedating Loeffler and Frosch's work on foot-and-mouth disease by several years. Iwanowski's filtrate was virulent after preservation ten months in 95 per cent. alcohol. Beijerinck[3] also found that the virus passes porcelain filters; he could find no organism in it, either microscopically or culturally. The virus can be dried and survives the cold of winter. Infection can be produced by dried leaves two years old, and Beijerinck thinks that it may be conveyed by dust. The virus is destroyed at 75° to 80° C., not by drying at 40° C. Minute quantities suffice to produce infection, 1 to 2 drops of virulent juices dropped on healthy plants. Beijerinck has interpreted his findings by regarding the infectious agent as a living fluid contagion, "contagium vivum fluidum."

A cultivation of the virus of Mosaic disease of tobacco and of tomato has recently been reported by Olitsky.[4]

1. Meyer, A.: Landwirtschaftliche Versuchsstationen, 1886, xxxii, 450.
2. Iwanowski, D.: Centralbl. f. Bakteriol. [etc.], 2. Abt., 1899, v, Orig. 250–254.
3. Beijerinck, M. W.: Bot. Centralbl., Beihefte, 1893, iii, 266. Centralbl. f. Bakteriol. [etc.], 2. Abt., 1899, v, 27–33.
4. Olitsky, P. K.: J. Exper. M., 1925, xli, 129–136.

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